Suitability of marine ornamental shrimp *Lysmata uncicornis* Holthuis and Maurin 1952 to commercial aquaculture and comparative performance with *Lysmata seticaudata* (Risso, 1816)

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Resumo

O *hobby* da aquariofilia deu origem a uma indústria multibilionária. Este é um facto irrefutável particularmente no caso da aquariofilia marinha, tendo-se assistido à criação de postos de trabalho motivada por esta atividade, desde pessoas que recolhem os organismos do meio ambiente, passando pelos vários intermediários associados ao embalamento, envio, transporte e em último caso, venda dos animais a importadores, grossistas ou retalhistas. Contudo, a componente marinha deste *hobby* e a indústria associada têm sido alvo de crítica por parte de conservacionistas que advogam a necessidade de mais espécies marinhas de entre as presentemente transacionadas serem cultivadas em cativeiro para dessa forma aliviar a pressão de pesca sobre as populações selvagens. Esta pressão na procura de soluções maios sustentáveis levou ao advento da aquacultura de espécies ornamentais. Entre as espécies comercializadas para aquários marinhos os crustáceos decápodes estão entre os organismos mais populares. Dentro deste grupo surgem os camarões limpadores, organismos muito populares devido às suas cores chamativas, morfologia bizarra e/ou delicada e comportamentos pouco comuns ou mesmo úteis num aquário marinho. O género *Lysmata* é um dos géneros de camarão ornamental mais popular por apresentar todas estas características citadas e uma adicional que os distingue e torna particularmente interessantes em cultura: o facto de serem hermafroditas simultâneos protândricos. O presente trabalho investigou a adequabilidade do cultivo de uma nova espécie de camarão ornamental *L. uncicornis*, uma espécie que apenas recentemente começou a ser transacionada para a aquariofilia, marinha. A fertilidade de reprodutores, a qualidade e desempenho larvar e o desenvolvimento de juvenis até atingirem o tamanho comercial foi avaliada do ponto de vista ´zootécnico e de exequibilidade económica da sua produção, tendo como termo de comparação o desempenho obtido no cultivo de uma outra espécie bem estabelecida neste mercado da aquariofilia marinha – *L. seticaudata*. *Lysmata uncicornis* mostrou fertilidade superior a L. seticaudata com cada indivíduo a gerar 753±440 larvas por libertação, L. seticaudata gerou 257±123 larvas por libertação. *Lysmata uncicornis* mostrou um desempenho inferior no teste de inanição com cada indivíduo a levar em média menos tempo a morrer, 5.5±1.8 dias, mais tempo a fazer a muda para Zoea II, 4.3±1.6 dias e menos indivíduos a fazer a muda para Zoea II, 142 de 300. Cada indivíduo de *Lysmata seticaudata* levou em média 7.1±1.5 dias a morrer, 3.4±0.7 a fazer a muda para Zoea II e das 300 larvas testadas, 268 fizeram a muda. Apesar do desempenho superior de *L. seticaudata*, a taxa de sobrevivência até pós-larva de L. uncicornis foi superior, 8.0±2.0% e o tempo passado na fase larvar, 25.3±0.6 dias, menor. *L. seticaudata* teve uma taxa de sobrevivência até pós-larva de 6.5±1.5% e passou em média 28.0±4 dias no estado larvar. Lysmata uncicornis levou 84.0±6.0 dias a chegar ao tamanho de venda com uma taxa de sobrevivência de juvenis de

61.0±15.1% e L. seticaudata levou 85.0±5.0 dias a chegar ao tamanho de venda com uma taxa de sobrevivência de juvenis de 67.3±3.8%. À luz dos resultados obtidos concluiu-se que é viável a aquacultura de ambas as espécies, considerando o custo de funcionamento, e é, de facto, exequível a produção de *L. uncicornis* em detrimento de *L. seticaudata.*

Palavras chave: Aquariofilia, espécies ornamentais, camarões limpadores, género *Lysmata, Lysmata uncicornis, Lysmata seticaudata,* hermafroditas simultâneos protândricos, exequibilidade económica, fertilidade de reprodutores, taxa de sobrevivência, pós larva, qualidade larvar, desenvolvimento de juvenis, tamanho de venda.

Abstract

The aquarium trade gave birth to a multi-billion-dollar industry. This is an irrefutable fact particularly in the case marine ornamental species trade with the activity generating numerous jobs from the individuals that collect the organisms from the environment to the various middlemen that carry out the packaging, shipping, transportation and, ultimately, the sales of these species to importers, wholesalers or retailers. Because of the trade's reliance on wild-caught marine species, the hobby and the industry have been heavily criticized by conservationists advocating the need for more captive-bred species among the ones currently in the trade so as to alleviate the pressure that fishing for these species puts on wild stocks. This pressure from conservationists and concerned members of the public eventually led to the dawn of marine ornamental species aquaculture. Amongst the traded species for marine aquaria, second only to fish and coral species, are the very sought-after decapod crustaceans within which ornamental cleaner shrimp are some of the most popular species due to their dazzling colors, bizarre and/or delicate morphology and unusual and even useful behavior. The genus *Lysmata* is one of the most popular ornamental shrimp genera in the trade, displaying every single one of these traits and one very distinctive characteristic that makes them that much more interesting for culture purposes: being protandric simultaneous hermaphrodites. The present work investigated the suitability to culture of *L. uncicornis*, a novel species in the trade. Broodstock animals' fertility, larval quality and performance and juvenile grow-out were assessed from zootechnical and economic feasibility standpoints using another well-established species, *L. seticaudata*, as comparison. *Lysmata uncicornis* showed greater fertility than *L. seticaudata* with each individual generating 753±440 larvae per release, *L. seticaudata* generated 257±123 larvae per release. *Lysmata uncicornis* showed a lesser performance in the starvation test with each individual taking less time to die, averaging 5.5±1.8 days, longer to molt to Zoea II, averaging at 4.3±1.6 days and fewer larvae molting to Zoea II, 142 out of 300 tested. Each *Lysmata seticaudata* individual took on average 7.1±1.5 days to die, 3.4±0.7 days to molt to Zoea II and out of the 300 larvae used in the test, 268 molted to Zoea II. Despite the superior performance of *L. seticaudata*, *L. uncicornis* showed a superior survival rate to post larva, 8.0±2.0%, and spent less time in the larval stage, 25.3±0.6 days. *L. seticaudata* displayed a survival rate to post larva of 6.5±1.5% and spent an average of 28.0±4.0 days in its larval stage. *Lysmata uncicornis* took 84.0±6.0 days to reach its market size (set at 25 mm) with a juvenile survival rate upon reaching it of 61.0±15.1%. *Lysmata seticaudata* took 85.0±5.0 days to reach the market size with a juvenile survival rate upon reaching it of 67.3±3.8%. In light of the obtained results it was concluded that it would be viable to produce both species simultaneously considering the operating costs. Furthermore, even if

charging the same for *L. uncicornis* and *L. seticaudata,* it would be possible to produce only *L. uncicornis* forsaking *L. seticaudata.*

Key words: Aquarium trade, ornamental species, cleaner shrimp, *Lysmata* genus*, Lysmata uncicornis*, *Lysmata seticaudata*, Protandric simultaneous hermaphrodites, Economic feasibility, broodstock fertility, survival rate, post larva, larval quality, juvenile grow-out, market size.

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Abbreviations List

- *L*. – *Lysmata;*
- RO - Reverse Osmosis;
- Ppt - Parts per thousand;
- PVC - Polyvinyl Chloride;
- LED - Light Emitting Diode;
- SWOT - Strengths, Weaknesses, Opportunities and Threats;
- EFA - Essential Fatty Acids;
- LOA - Linoleic Acid;
- LNA - Linolenic Acid;
- ARA - Arachidonic Acid;
- EPA - Eicosapentaenoic Acid;
- DHA - Docosahexaenoic Acid;
- PUFA - Poly-Unsaturated Fatty Acids;
- HUFA - Highly Unsaturated Fatty Acids.

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1. Introduction

1.1. Aquarium trade

The fascination with marine or aquatic life can be traced back to ancient civilizations. As early as 2000 BC, the Egyptians, Assyrians and Chinese kept fish for the purpose of having a readily available food source and for religious reasons. In ancient China and Egypt fish started to take on a decorative and ornamental role and likely became status symbols. During the first century BC wealthy Romans continued this trend with the *vivarie piscinae,* elaborate sea-side pools, where they kept live seafood. Eventually the pools took on the added purposes of entertainment, becoming homes for aquatic pets, status symbols and social gathering sites. It was the Chinese, however, that forged the path to the ornamental culture and trade with literature suggesting that they identified ornamental phenotypes of carps (*Carassius auratus*) nowadays known as the common gold fish (Palmtag, 2017).

The aquarium enthusiasts have, since then, led to birth of the aquarium trade, a very successful industry that moves millions worth of fresh and saltwater species over the world (Figueiredo and Narciso, 2006; Palmtag, 2017. Although marine species make up less than 10% of global ornamentals trade, in terms of value the percentage is much greater and ever increasing. Southeastern Asian countries (mainly Indonesia and the Philippines) (Corbin et al, 2003) supply most of the traded marine ornamentals that are then exported to North America, Europe, Japan and more recently, China as well (Palmtag, 2017). A potential drawback for most marine ornamental aquaculture ventures is that with these being based mostly in the USA and EU countries, there is a lack of sharing of developed knowhow with exporting countries that directly impairs these generally impoverished regions. To implement such aquaculture practices could come as an alternative income source for the people (Calado, 2008a). For both fresh water and marine species, fish are the more prevalent group of animals

being traded. However, the last 25 years have seen a rise in interest in miniature reef ecosystems, as opposed to fish-only aquariums. This motivated demand for various invertebrate species associated with coral reefs (e.g., anemones, jellyfish, starfish, urchins, mollusks and decapod crustaceans) besides the corals themselves (Palmtag, 2017). An estimate of 1800 fish species, along with 700 invertebrate species, including but not limited to cnidarian, mollusk, arthropod, echinoderm, annelid, and poriferan species, are traded today (Palmtag, 2017) with reef fish and corals being the most traded organisms (Calado, 2008a). Aside from corals, the most desired invertebrate group for marine aquariums are decapod crustaceans (Calado et al, 2003a). Ornamental species receive their ornamental status based on their physical traits such as flashy coloring or delicate and exotic body shapes (Figueiredo and Narciso, 2006) but, also, due to certain desirable behaviors (such as associative behaviors) they may display. Cleaner shrimps, for example, help maintain the aquarium and the species present there in good health (Olivotto et al, 2011). Resilience in captivity (cultured animals are accustomed to captive conditions and more resilient than wild animals, additionally, hobbyists may be willing to pay extra for higher quality livestock) (Figueiredo and Narciso, 2006), not being harmful to other species in the aquarium (also known as being reef-safe) (Calado et al, 2003b) and being able to help keep in check certain undesirable species (Calado e Narciso, 2005) are other traits that grant a species its ornamental status.

The journey of a marine ornamental can involve the hands of many middlemen (Olivier, 2003), starting at the artisan collector's hands for those species not cultured to later be sold to a wholesaler (Olivier, 2003), should the collector not work for a specific wholesaler. Sessile species and live rock are simply removed from their location, while motile species may require snorkeling gear, underwater breathing devices and tools the like of nets, slurp guns or sedatives (Palmtag, 2017). The animals are then stockpiled and prepared for export. The wholesaler either sells the livestock to an exporter or acts as one. Export typically requires government-mandated permits specific to the locale and import destination, veterinary inspection, treatment and packaging of said livestock to prepare it for an international flight headed to the import company's destination (Olivier, 2003). When the shipment arrives on destination, the importers unpack the livestock, inspect it and acclimatize it to a temporary holding facility. At this point the livestock may be repacked for shipment to another destination, sold to a wholesaler, or directly to a retail outlet (Olivier, 2003; Palmtag, 2017). Wholesalers may also acclimate the livestock and either sell it to retailers or directly to home aquarists via the internet. Public aquaria can also do this or buy directly from importers, whereas home aquarists typically buy their livestock from retail outlets with a physical store or importers, wholesalers and retailers with online shops (Palmtag, 2017). Transshipping came up as a way to cut middlemen. It had its inception during the 1970's and consists in the amassing of

shipment orders submitted by a consortium of retailers (or wholesalers) at the import location by the transshipper. The order is then placed to an exporter and distributed to the consortium upon its arrival. There are however no guarantees that the livestock will reach the purchaser alive when distributed this way with the purchaser generally having to pick up the shipment himself. Transshipping has thus been the subject of studies and work geared towards improving the survivability of the livestock (Olivier, 2003; Palmtag, 2017). Figure 1 better illustrates this complex trade chain.

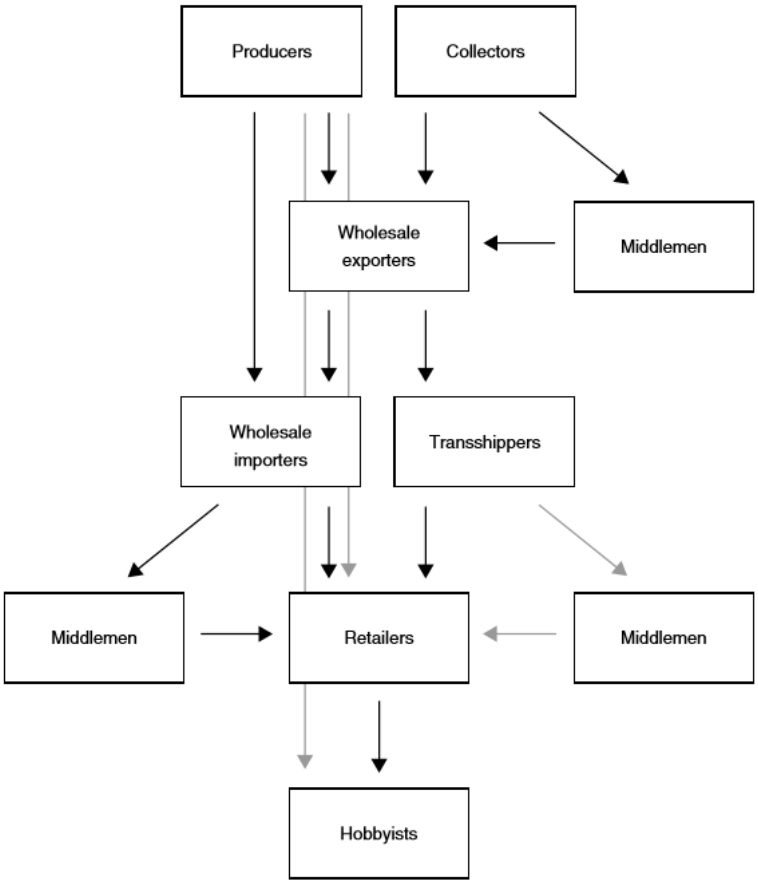

Figure 1: Path of a marine species in the marine ornamental trade. Grey arrows mark less common trade practices (adapted from Calado R., 2008 Introduction. In: *Marine Ornamental Shrimp Biology Aquaculture and Conservation*).

Much progress has been made in the last couple of decades in the aquaculture of marine ornamental species with an encouraging number of new species being introduced to the trade, however it is likely that most of these will not soon be introduced to the market at a viable price (Calado, 2017). The industry's interest in decapod crustaceans, motivated by the demand for these animals, is not necessarily recent and, within this group, there are high value species such as reef lobsters and certain shrimp. The problem for these resources, when captive bred, arises during these animals' larval rearing (Calado et al, 2005a) and maturation process. Therefore, the relative lack of success from commercial scale culture efforts lead to a considerable pressure on wild populations so that the demand for these species can be met.

Despite efforts made towards rearing these animals in captivity, the large majority (over 90%) of species currently traded are still wild caught (Corbin at al, 2003; Calado et al, 2005a; Olivotto et al, 2011) with less than 10% of traded species being captive bred (Palmtag, 2017) in a strong contrast to what happens with traded freshwater fish species of which about 90% are captive-bred (Palmtag, 2017). This situation poses reservations on these species' trade owing to the ecological implications that overfishing, particularly if conducted in an unregulated and unsupervised manner (Calado and Dinis, 2008), can carry, for instance, on the species associated to the cleaner shrimps of the genus *Lysmata* and, obviously, on the shrimp species themselves. However, it should be noted that these impacts are, for the most part, yet to be clearly listed (Calado et al, 2003b; Calado et al, 2009). What is known is how certain capture methods (cyanide and bleach usage and coral breaking and smashing for example) are highly damaging to the coral reef (Olivier, 2003; Bunting at al, 2003; Calado, 2008a) where these animals dwell, thus contributing to the direct depletion of target species (Calado et al, 2003b; Palmtag, 2017) and, with abundant evidence linking it to coral bleaching and mass mortality of non-target species (Palmtag, 2017). The destruction of already threatened coral reefs, be it from climate change, water pollution or other anthropogenic factors, can be further magnified by unsustainable fishing practices (Calado, 2008a). Increased mortality of species harvested using cyanide fuels a sinister cycle of habitat destruction and increased demand to replace lost livestock due to high post-collection mortality (Palmtag, 2017). Captive cultivation of these animals presents itself as a viable option to both remove pressure from wild stocks (Calado, 2008a) and even perform restocking efforts. Life history and other basic scientific knowledge are still unknown for many of these species, with broodstock management, spawning induction, larval rearing, nutrition requirements, live feed, diseases and suitable culture systems being some of the bottlenecks for these species' commercial scale production (Calado, 2008a; Palmtag, 2017; Rhyne et al, 2017). While it is naturally desirable that the industry shifts from wild caught to captive-bred, this does not mean that collection should be outlawed, provided it is done sustainably and for those species that can endure the collection effort (Calado, 2017).

Amongst marine ornamental decapods none are more popular than marine ornamental shrimp (Calado et al, 2005a; Figueiredo and Narciso, 2006; Calado, 2008a). What then gives an ornamental shrimp its ornamental status? Dazzling coloration, bizarre and/or delicate external morphology, unusual reproductive traits (such as protandric simultaneous hermaphroditism) (Bauer and Holt, 1998; Palmtag and Holt, 2007), symbiotic and unusual behavior, fish cleaning services, control of nuisance organisms (like the anemone *Aiptasia pallida*) and being reef-safe (not harming other organisms in the aquarium) (Calado, 2008a) are the traits that prompt aquaculturists to recruit a shrimp species as ornamental. With coloration being the most

common trait used, this is followed by distinctive morphology like modified chelae, symbiotic behavior (e.g., shrimp associations with cnidarian species like anemones or with fish species like gobies and moray eels). These specimens have been long time favorites of reef aquarium keepers (Calado, 2008a). Unusual behavior like the side-to-side movement akin to a rocking dance cleaner shrimp engage in when signaling their cleaning services (Becker et al, 2005) that they then perform with their multi-articulate clawed second pair of pereiopods, thoroughly inspecting the fishes' skin, mouth and gills after they are allowed to climb on (Calado, 2008a) and the ability to control pest species, thus allowing a shift from technological solutions to biological ones as far as these issues are concerned (Calado, 2008a), make these animals interesting to aquarium keepers. Most decapod crustaceans present both sexes (Bauer and Holt, 1998; Calado, 2008a) individually with some being sequential hermaphrodites, starting their lives as males but eventually, as they mature, becoming females. This form of hermaphroditism, known as protandry, has been well documented particularly within carideans (Bauer and Holt, 1998). However, it has been found that the genus *Lysmata* has a very unique form of hermaphroditism, protandric simultaneous hermaphroditism, meaning than even after changing from the male phase to the hermaphrodite phase, the animals still retain the ability to fertilize one another (not being able to self-fertilize) effectively meaning that both members of the breeding pair can become ovigerous (Bauer and Holt, 1998, Palmtag and Holt, 2007; Calado, 2008a). This coupled with unusual mating patterns and reproductive traits the likes of monogamic behavior with the animals still recognizing their mated pair even after considerable time apart and crowds with large dominant males that keep harems and display agonistic behavior towards smaller males have earned these animals their place in reef aquaria (Calado, 2008a).

The most traded species of marine ornamental shrimps belong to the families:

- *Stenopodidae,* these animals, generally seen in shallow water are, curiously, not even true shrimps as evidenced by the chelae in the third pair of pereiopods rather than the second and may be ancestors of the so-called true shrimps, these are some of the most traded animals with the most popular being the species *Stenopus hispidus* (Calado, 2008b).
- *Alpheidae,* commonly known as pistol shrimps, these are promptly distinguishable from stenopodideans by the lack of chelae in the third pair of pereiopods. With at least 36 genera only members form the genera *Alpheus* and *Synalpheus* are available in the aquarium trade. The most distinctive characteristic of these animals are their snapping modified chelae (used for defense or aggression) that can produce a loud sound akin to that of cracking glass. These animals are also known to associate with other species like cnidarians, mollusks, fish and other crustaceans (Calado, 2008b).

- *Gnathophyllidae,* commonly displaying stout small bodies and associating with echinoderms, these tend to be avoided by aquarium keepers due to the need of a specialized tank to properly house them. Commonly known as bumblebee shrimps due to the transverse black, white and yellow bands their bodies display (Calado, 2008b).
- *Hymenoceridae,* shrimps dubbed as harlequins are currently in the family *Hymenoceridae*, though previous literature refers to them as members of the *Gnathophyllidae*. These shrimps are some of the most amazing decapods in the world, displaying unique morphological features and coloration. They are promptly recognized by divers and aquarium keepers, and have been compared to orchids and mounted knights in ornate armor. Due to their monogamic behavior and agonistic behavior towards conspecifics of the same sex, a mated pair of harlequin shrimps reaches higher market values than individuals sold separately (Calado, 2008b).
- *Palaemonidae* (subfamily *Pontoniinae*), the most highly sought-after shrimps in the family *Palaemonidae* are the partner shrimps of the genus *Periclimenes*. This genus belongs to the subfamily *Pontoniinae* and, like most other genera in the subfamily, displays associative behavior with many invertebrates (cnidarians, mollusks and echinoderms) and fish species as well. In fact, given the obligate nature of some of these associations and the technical obstacles to keeping certain invertebrate hosts in captivity, many *Periclimenes* species cannot be kept in marine aquaria (*P. imperator* for example given its association with the Spanish dancer nudibranch *Hexabranchus sanguineus*). (Calado, 2008b).
- *Palaemonidae* (subfamily *Palaemoninae*), another group of shrimps has started to be traded in the aquarium industry, are the members of the genus *Urocaridella*. These shrimps belong to the subfamily *Palaemoninae*. Unlike partner shrimps, *Urocaridella* species show a very long rostrum and their translucent bodies are covered by small and numerous red, white, yellow or brown patches. Although most of the specimens traded are usually sold as *Urocaridella antonbruunii*, the taxonomy of *Urocaridella* species is yet unclear (Calado, 2008b).
- *Rhynchocinetidae,* commonly known as hinge beak shrimps, their unusual way of locomotion, resembling tango dancers, has also granted them the popular name of dancing shrimps. Unlike other shrimp species, they can move the rostrum vertically because of a flexible joint between it and the carapace. The most commonly available species is *Rhynchocinetes durbanensis,* characterized by strong sexual dimorphism, with dominant males exhibiting the first pair of chelipeds greatly enlarged (Calado, 2008b).

- *Hippolytidae,* probably the most popular ornamental shrimps among aquarium hobbyists, the family includes the genera *Lysmatella* (*Lysmatella prima* is closely related to the species of the genus *Lysmata* though easily differentiated by the absence of epipods – structures employed in gill cleaning inside the branchial chamber – on the pereiopods of *Lysmatella* individuals. Total length of up to 3 cm with white to yellowish legs, long antennae and chocolate brown longitudinal stripes. Though rare in the trade it is a very sought-after species), *Parhippolyte* (the species *Parhippolyte mistica* reaching up to 4 cm total length is a cryptic species present in Indo-Pacific marine caves. Its third to fifth pereiopods are white and remarkably long and its abdomen and telson show characteristic vertical red bands. The antennae are also very long, being red in their anterior tip and progressively turning brilliant white towards their posterior end. Due to their long pereiopods, *P. mistica* appears to gently hover over the bottom, an interesting trait for aquarium keepers), *Saron* (commonly named marbled shrimps, the most readily available member of the genus is the species *S. marmoratus* (Olivier, 1811), found from East Africa to Hawaii, its marbled look is due to patches of various brownish hues over the entire body along with numerous bush-like setae on the body. Reaches up to 5 cm) and *Thor* (*Thor amboinensis* (De Mann, 1888) or sexy shrimp as it is commonly known earns its name and ornamental status due to its associative behavior with numerous species of sea anemones and its habit of stretching and waving the abdomen upwards. The reason for this unusual behavior is still unknown. Small species with the adults reaching up to 2 cm, the animals display transparent brownish bodies dotted with large iridescent white dots bordered by a thin blue line. One of the few truly circum-tropical species) (Calado, 2008b).
- *Lysmatidae* (Calado et al, 2017)*,* the family includes genus *Lysmata*, arguably the most popular genus in the trade. Protandric simultaneous hermaphrodites (Bauer and Holt, 1998; Palmtag and Holt, 2007), these shrimps, commonly known as peppermint and cleaner shrimps, display average body sizes ranging between 3.5 and 6 cm. These shrimps belong to a large number of distinct *Lysmata* species, although many of them are thought to be closely related. Despite species-specific differences in their coloration, all peppermint shrimp species generally display semi-translucent bodies with longitudinal, transverse, and/or oblique red bands. Certain species display a more or less intense blue tinge on the posterior ends of their telson and uropods. All these species are generally labelled as ‘wurdemanni’ in the trade and are commonly used in marine aquariums to control the outbursts of glass anemones *Aiptasia*. (Calado, 2008b).

From the species in the trade belonging to these families, the decapod crustaceans from the genera *Enoplometopus*, *Hymenocera*, *Lysmata* e *Stenopus* are among the most highly prized and, also, highly priced (Calado et al, 2003b; Calado et al, 2005a; Figueiredo and Narciso, 2006). Species the likes of *L. seticaudata* (Risso, 1816), *L. amboinensis* (De Man, 1888) *L. debelius* (Bruce, 1983) or *L. wurdemanni* (Gibbes, 1850) have thusly been the subject of many studies geared toward their mass production in a captive bred regime for commercialization. To this end, the animals of the genus *Lysmata* display a set of characteristics that, paired with their flashy and colorful looks, makes them particularly interesting for the aquarium trade. The fact they are protandric simultaneous hermaphrodites (Palmtag e Holt, 2007) (they start their lives as males and eventually develop the female reproductive system aside the male one) makes the production that much more lucrative since both members of the reproductive pair can become ovigerous (Calado et al, 2005b). Furthermore, these are cleaner shrimps which means their activity and behavior can benefit the other species present in the aquarium and they also help keep in check an undesirable anemone species, *Aiptasia pallida*, (Calado e Narciso, 2005; Calado, 2008b) that can be introduced in the aquarium through the use of the so called "live rock" (rocks cultivated in such a way that they are not entirely devoid of life or harvested from the environment) (Olivotto et al, 2011) since they feed on these anemones. The present work will be specifically focusing two species of marine ornamental shrimps from the genus *Lysmata*.

1.2. Species Overview

The species that were used in the present thesis were *Lysmata seticaudata* (Risso, 1816) and *Lysmata uncicornis* Holthuis and Maurin 1952*,* are both members of family *Lysmatidae*. *Lysmata seticaudata* exhibits large hermaphrodite individuals, reaching up to a total length of 67 mm. The body is robust. The rostrum is short and does not extend beyond the antennal peduncle, it bears six dorsal and two ventral teeth and well-developed eyes. The carapace is smooth and its latero-anterior edges are adorned with a strong antennal spine and a small pterygostomian spine and the first pair of pereopods ends in a pair of pincers with its carpal segment being shorter than the pincer (Lagardère, 1971). The living specimens bear beautiful shades of red, lighter or darker depending on the color of the substrate. The chromatophores are distributed in a very characteristic manner creating lightly colored longitudinal stripes extending over the carapace and spanning over all the abdominal segments. This species occurs frequently in the residual pools created by low tides, lying under the overhangs or in the crags of the algae covered rocks. These animals can also descend to a few meters deep. In the Mediterranean, these animals have been found between 0 and 15 meters in depth in

rocky areas with algal cover and in *Posidonia* meadows. This latter location would be particularly relevant to juvenile shrimp. *L. seticaudata* hunts at night in the *Posidonia* meadows and on the detritus near the rocky areas feeding on polychaetae, other animals' larvae, eggs and animal detritus. During the day, the animals withdraw to the shelter of the *Posidonia* rhizomes or crags and hollows in the rocks. These animals are protandric hermaphrodites and the main phases of the biological cycle of this shrimp are as follows. The coupling is generally unique, in cross, fertilization is internal and occurs throughout the period between March and October and the eggs are laid during the night following mating. The female then retreats into the rocky areas. The number of eggs emitted varies between 150 and 500 and embryonic development lasts two weeks. A gradual change in the color of the eggs from orange yellow to greyish green is observed and this last hue indicates that the release of the larvae should be very close.

The larvae, during the summer, live in deep plankton a few kilometers off the coast. They stay there for about two months and make nine molts, one molt per larval stage if no mark time molting occurs. At this point the animals start to gradually return to the coastal regions to lead a benthic life. *L. seticaudata* is considered an adult after three months for a size of 10 to 11 mm and can reach up to the age of four years for a size of 65 mm.

The larval gonad is divided into two parts, one testicle (caudal half) and one ovary (cranial half). *L. seticaudata* will go through three consecutive sexual phases during its life. The male phase begins immediately after the end of larval life and young males are able to mate in the spring of their first year of life. The phase of transition appears from March to April and affects males whose size is between 31 and 51 mm. It is thus relevant, approximately, in the middle of the life of the individual. Lastly, the female phase, will continue until the end of the animal's life. In the wild, the appearance of this phase occurs from June or July (for a minimum total length of 30 mm) until November and the new female performs two to three spawns. During the Winter of the second year a five-month sexual rest is observed after which the animals resume egg production somewhat earlier (Lagardère, 1971). *Lysmata seticaudata* ranges from the East Atlantic off the coasts of Europe (further North than *L. uncicornis*) and Morocco, where it inhabits the Rabat-Casablanca sector, meaning that these animals may share a habitat with *L. uncicornis* (Jean-Paul Lagardère, 1971; Ricardo Calado, personal communication), to the Mediterranean shores (Jean-Paul Lagardère, 1971). Figures 2 and 3 showcase adult individuals.

Figure 2: Wild caught *Lysmata seticaudata* broodstock before being introduced to the tanks. On the bottom left corner, it is possible to see a *L. uncicornis* individual (photo taken by André Rocha).

Figure 3: *Lysmata seticaudata* individual in a maturation tank (photo taken by André Rocha).

L. uncicornis individuals can measure up to 53 mm total length, displaying well developed eyes and a straight rostrum that reaches or slightly surpasses the middle of the second segment of the antennal peduncle. The extremity of the rostrum exhibits a slight upward curve, its upper edge bears six to seven teeth and its lower edge two or three. The latero-anterior edges of the carapace are adorned with a strong antennal spine but there is no trace of the pterygostomian spine (Lagardère, 1971). The first pair of pereopods ends in a pincer with its carpal segment being the same length of the pincer. Live specimens exhibit a beautiful red color, diffuse on the carapace and arranged in transversal stripes with a clear contour on the abdomen. A red circular patch, very characteristic, is observed on the sixth abdominal segment. This species appears to be less coastal than *L. seticaudata* thus being found on rocky bottoms still immersed in the company of the common prawn *Palaemon serratus*. This species, like the rest of the *Lysmata* genus, displays protandric hermaphroditism with its life cycle being similar to that of *L. seticaudata*. Its laying period extends from December to April, with a maximum in January-February with the females typically having a size between 30 and 45 mm during this season. (Lagardère, 1971). However, in captivity and under the right conditions these animals become continuous breeders should one replicate tropical water temperature (Ricardo Calado, personal communication). The time in between molts is 25 days at a temperature of 16 ° C. During breeding season, the absence of algal food causes necrosis of certain appendages and the rather rapid death of the animal. This species geographical distribution was described as the Atlantic coast of Morocco (rocky bottoms in the Rabat-Casablanca sector) (Lagardère, 1971) but these animals are now being found further North, off the coast of Algarve in Southern Portugal (Ricardo Calado, personal communication). Despite all that has been exposed above, this species remains somewhat of a novelty item in the aquarium trade and some problems, namely its acclimation to captivity, remain relevant (Ricardo Calado, personal communication). Figures 4 and 5 showcase adult individuals.

Figure 4: Wild caught *Lysmata uncicornis* broodstock before being introduced to the tanks. Notice how the individuals display mature gonads and some are already incubating embryos in their abdomen (photo taken by André Rocha).

Figure 5: *Lysmata uncicornis* individual in a maturation tank (photo taken by André Rocha).

1.3. Considerations on Broodstock Husbandry and Maturation System

A simple, easy to use, recirculated system must be selected for the husbandry of broodstock animals that will mature and breed in captivity, thus, providing larvae for culture. The system must display a series of tanks divided in two chambers by means of a plastic mesh, connected to a sump equipped with the necessary devices to insure recirculation and proper water quality. This set up allows to avoid previous methods, such as the capture of ovigerous females that would then be moved to a basket inside a separate tank where they would release the larvae to be returned to the breeding tank. These methods cause needless stress to the animals that can interfere with the release and molting processes and, more importantly, in the case of species that display strong agonistic behavior towards their conspecifics (this has been well documented for the genus *Lysmata*), separating the breeding pair can interfere with pair-bonding and lead to aggression upon returning the female or euhermaphrodite to the breeding tank which can ultimately result in loss of broodstock animals.

A recirculation system should be preferred since it helps to standardize water quality across the tanks something that would not happen if an approach using separate tanks was chosen, namely because the ratio biomass/water volume would likely be significantly higher thus more easily allowing for fluctuations in the water quality parameters.

The way the system is set up also makes routine tasks such as filter cleaning, tank siphoning, larvae extraction and water quality assessing easier to handle (Calado et al, 2007).

1.4. Considerations on the Larviculture System and its Constraints

Larviculture of the genus *Lysmata* has been pointed out as the main bottleneck impairing the successful commercial scale cultivation of the genus in captivity (Calado et al, 2003a; Calado et al, 2003b; Calado et al, 2008). Aquaculture engineering provided the means to solve these problems when coupled with the work of larval morphology specialists that helped ascertaining the unsuitable rearing conditions allowing aquaculturists to properly address them (Calado, 2008). This allowed for encouraging success in small scale breeding of these animals in laboratories with the prospect of making commercial scale culture viable, namely, using recirculation systems employing cylindrical tanks with spherical bottoms as an alternative to the previously used cylindrical cones. These new tanks, in keeping with what Greve's "planktonkreisel" system (1968) achieved, assure a good larvae suspension and food availability (since these larvae's ability to feed depends on chance encounters with the prey item) through the upwelling water motion (Calado et al, 2003a) and thus avoiding other suspension methods, such as aeration, (Ricardo Calado, personal communication) that can

damage certain structures of some larval stages, namely the paddle shaped appendages that one can observe from the second zoeal stage onwards in *Lysmata* larvae (Calado et al, 2004). Adopting a recirculation system provided the larvae with the same advantages it did the broodstock by standardizing water quality and streamlining routine tasks, thus reducing stress to the animals.

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Nutrition during the larval stages comes up as a very important topic to address if one is to ensure a good survival rate to the post-larva stage. Supplying an adequate diet is key during larval development. Just after hatching these animals are facultative primary lecithotrophs, this means that they are born with vitellogenic reserves that they can use if no food is available (Ricardo Calado, personal communication). It was common practice to starve out the larvae in their zoea I stage during their first day of life on the working assumption that the reserves they hatch with were enough to ensure a successful molt into the zoea II stage (Simões, Ribeiro e Jones, 2002). However, this practice can lead to mark-time molts (molts with little to no changes in morphology even if exhibiting an increase in size), something usually associated with an inadequate nutrition (Calado et al, 2005a). Despite this possibility the animals can generally make the change to Zoea II with no food. More relevant is the concept of facultative secondary lecithotrophy (Calado et al, 2007), this means that the animals during their larval stages are capable of accumulating reserves in their hepatopancreas that they can then use during their later larval stages if the exogenous food availability is suboptimal (Calado et al, 2007). This ability, along with a less than ideal nutrition, allows the animals to extend their larval stage through mark-time molts until a time at which the ideal conditions are reestablished. Mark-time molts are a particularly relevant problem to the culture effort because the phenomenon is liable to contribute to a poorly synchronized settlement of competent larvae that will, in turn, enable the interaction between newly metamorphosed specimens and juveniles of different sizes and, therefore, increasing the risk of cannibalism, a phenomenon that has been well documented among decapod crustaceans (Calado, 2008b; Calado, 2008c; Calado et al, 2017).

1.5. Objectives

The goal of the present study to ascertain whether the species *Lysmata uncicornis,* somewhat new to the aquarium trade, could be feasibly mass produced for commercialization to both private hobbyists and public aquaria, when compared to *L. seticaudata*. To that end, a series of hypotheses pertaining to larval quality, volume of larvae produced by broodstock animals, larval survival and performance and juvenile survival and performance were formulated. The following null hypotheses were tested in the present study:

- there are no significant differences between the average number of larvae generated per animal between the two species;
- there are no significant differences between the two species in the average time it takes for a starved-out larva to die;
- there are no significant differences between the two species in the average time it takes for a starved-out larva to undergo the first metamorphosis into Zoea II;
- there are no significant differences between the average survival rates to the post larval stage between the species;
- there are no significant differences between the average time spent in the larval stage between the species;
- there are no significant differences between average survival rates upon reaching market size between the species;
- there are no significant differences between the average time it took for the animals to reach market size (from larval hatching to juveniles reaching a commercially suitable size).

2. Material and methods

2.1. *Lysmata spp.* Aquaculture

2.1.1. Broodstock Husbandry and Maturation

The breeding pairs' maturation system was set up according to what was described in Calado et al, 2007 with 24 maturation tanks (0.50 m long, 0.15 m wide, 0.22 m tall with a total volume of 16.5 L), twelve with two shrimp each and the upper level being used to raise juveniles during maturation to commercial size with a 415 L total volume sump (1.90 m long, 0.4 m wide and 0.55 m tall) equipped with two 7000 L h^{-1} pumps (one for each level), a protein skimmer, a filtration bag to ensure that no siphoned food ended up in the sump and media (bioballs) to ensure biological filtration. Figures 6 and 7 illustrate the maturation system employed.

An osmorregulator was fitted to the sump by installing a water level sensor inside the sump connected to a console that, should the water level fall below the desired value, would trigger a pump inside a plastic drum filled with Reverse Osmosis (RO) water until such time as the desired level had been reestablished and thus keeping the salinity at the desired value – 35 parts per thousand (ppt). Figures 8 through 10 depict this.

Figure 6: Broodstock maturation set up to the left. Notice the large sump underneath (photo taken by André Rocha).

Figure 7: Sump detail depicting the skimmer, filtration bag, biological media bags and the outflow tube (to the back) connected to the pump that ensured recirculation (Photo taken by André Rocha).

Figure 8: Water level sensor (photo taken by André Rocha).

Figure 9: Osmorregulator console (photo taken by André Rocha).

Figure 10: Plastic tank containing the freshwater purified by reverse osmosis and the osmorregulator pump (photo taken by André Rocha).

The artificial salt water used during the experiment was prepared using RO water and mixing 35 g of Pro Reef™ pharmaceutical grade sea salt for every liter of RO water, thus achieving

the desired salinity of 35 ppt. The tanks were set up in parallel according to a recirculation system, as previously stated, with water being distributed to the tanks through 25 mm diameter polyvinyl chloride (PVC) pipes. A valve was fitted to the water inflow pipe (10 mm in diameter) to regulate the water flow ensuring the any larvae released would be carried through the movable 5 mm^2 plastic mesh placed close to the front glass of the tank (about 1/5 into the tank) to prevent cannibalism by the adult shrimp. Beyond this mesh, also in the front glass of the tank, was the outflow opening fitted with a pipe (10 mm in diameter) that connected the tanks to the drainage tube that returns the water to the sump. On the inside of the tank a PVC T-shaped tube was connected and fitted with a fine mesh to prevent loss of larvae and to avoid the passage of uneaten food and other particles to the sump, the longer arm of this T-shaped tube pointed upwards with its opening and lined up with the edge of the tank thus preventing any spill should the mesh get plugged by uneaten food remains or by the animals' waste. This opening also regulated the water column in the maturation tanks keeping it at 0.20 m and an approximate volume of 15 L per tank. Blue LED lights were fitted outside the front glass of each tank close to the bottom to aid the flow in getting the larvae to go through the mesh, given the fact that they exhibit a positive phototactic response. The animals were kept on an inverted photoperiod (from 10:00 to 22:00 lights would go out and come back on from 22:00 to 10:00) to allow the operator to work during the darkness hours a red light would be kept on since these animals cannot detect the red wavelength. Figures 11 through 14 illustrate this.

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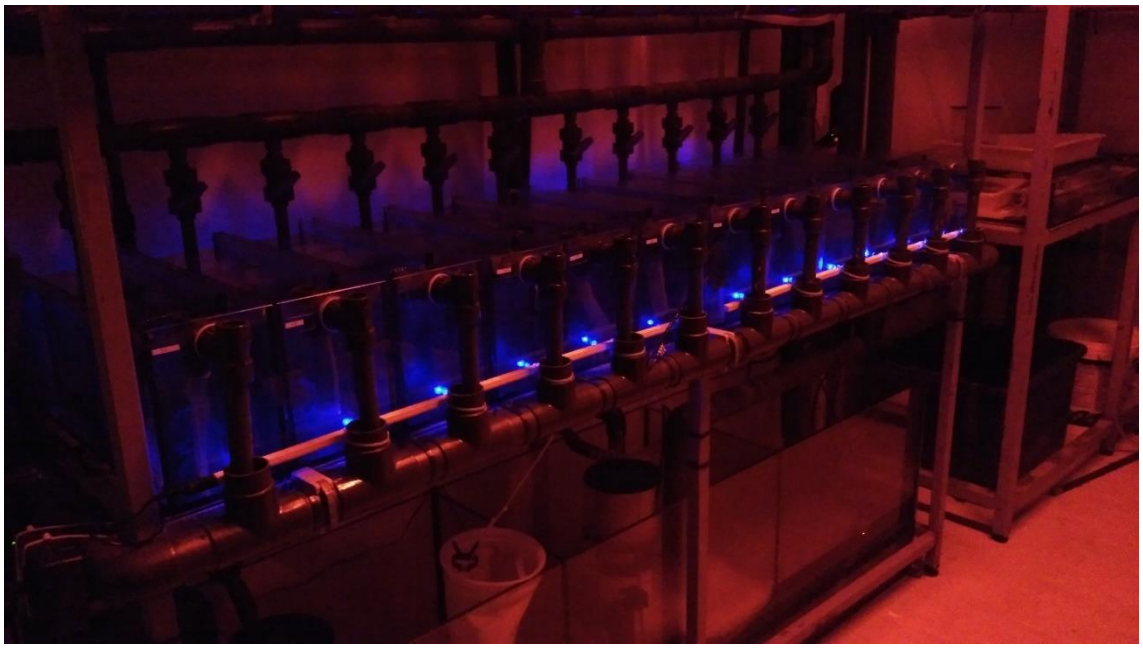

Figure 11: Broodstock maturation set up. Notice the blue LED lights meant to attract the larvae upon hatching and the red lighting meant to replicate hours of darkness while allowing for the operator to work (photo taken by André Rocha).

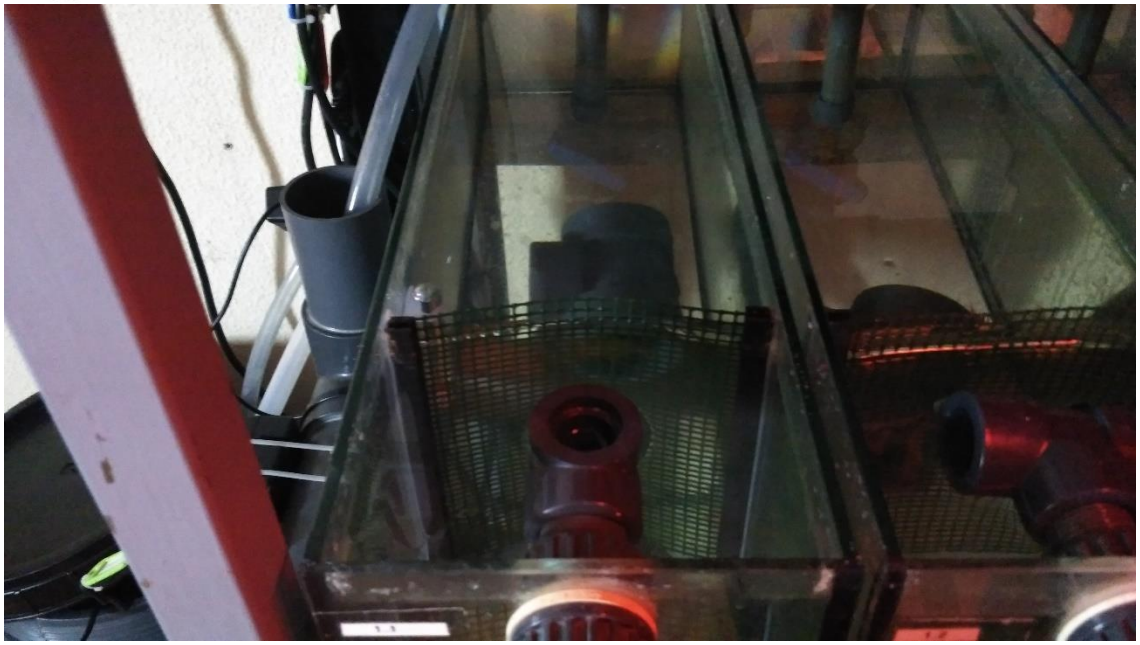

Figure 12: Green plastic mesh barrier to prevent the adults from preying on the larvae. Notice the inflow pipe to the back and the T-shaped tube to the left where the mesh filter would connect (photo taken by André Rocha).

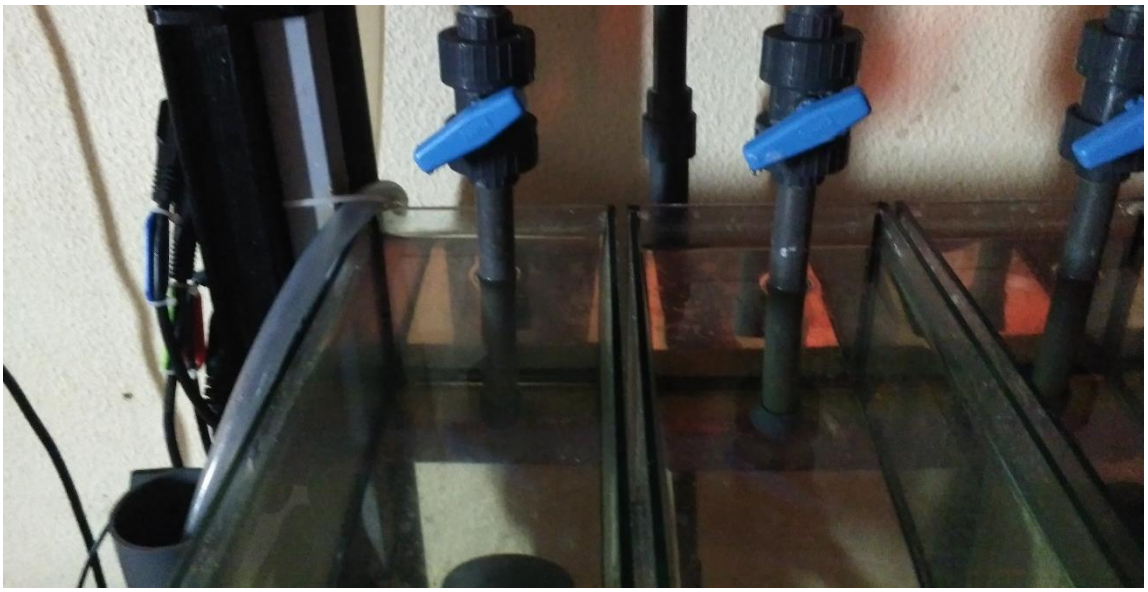

Figure 13: Valves fitted to the inflow pipes (photo taken by André Rocha).

Figure 14: Fine mesh filters that connected to the T-shaped tubes in the front of the tank to prevent loss of larvae and food from passing into the sump (photo taken by André Rocha).

The breeding pairs were fed 5 times a day (each feed totaling about 10% of the animals' live weight) with two-hour intervals in between each feeding. The first feeding was half a scoop of Hikari™ store-bought formula feed and the subsequent four feedings were what is designated as a practical feed, this is to say, a feed whose exact composition and nutritional value is not precisely known but that is sure to bring about the desired traits in the animals fed with it (Ricardo Calado, personal communication), gonad maturation and egg production in this case. This practical feed that was just mentioned was comprised of a seafood mix (comprised of shrimp, mussels and clams) commonly used to cook seafood rice and paella. Uneaten food was siphoned out. Figures 15 and 16 depict the feeding items.

Figure 15: Hikari™ shrimp formula feed (photo taken by André Rocha).

Figure 16: Seafood mix practical feed (photo taken by André Rocha).

Before larviculture trials could begin, considering that wild caught breeding pairs were being used, it was important to make sure that these animals were maturing their gonads and generating eggs and embryos mobilizing the nutrients of the diets being provided in captivity (Ricardo Calado, personal communication). To that end, the first three releases were not considered for the study.

2.1.2. Larval Quality and Larviculture

A starvation trial, geared towards ascertaining the fitness of the generated larvae, was conducted. This trial consisted of the starving of samples of 60 larvae per species with a total

of five replicas for each of the species. A total of ten SARSTEDT™ plastic six-well specimen plates, labeled with the maturation tank number, from A to J and the wells from 1 to 6 in roman numerals, were used and one larva was introduced in each well (approximately 10 mL in volume), then left to starve. The data were tallied every day until every one of the larvae was deceased. A total of 300 larvae was used in these trials per species. Figures 17 and 18 show the specimen plates and their tagging.

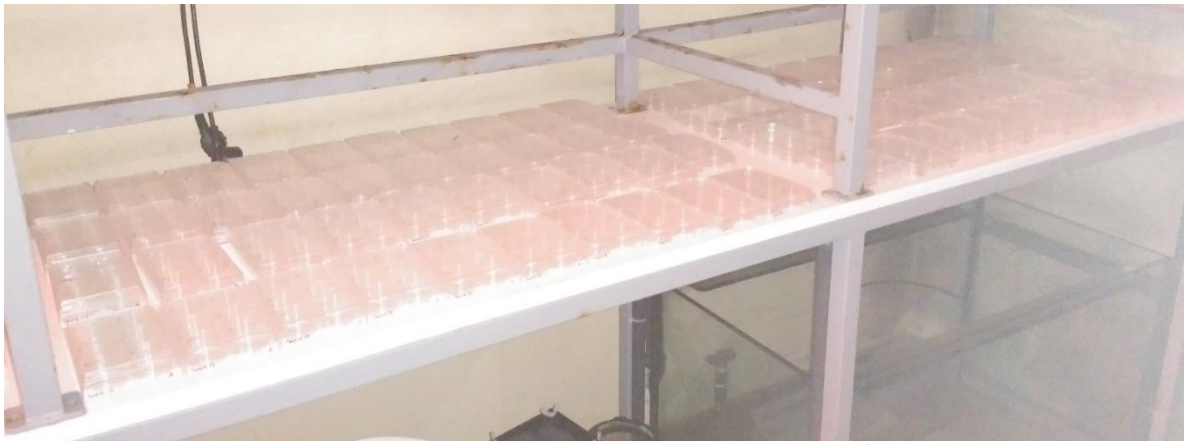

Figure 17: Specimen plates. Each row pertains to a 60 larvae replica (photo taken by André Rocha).

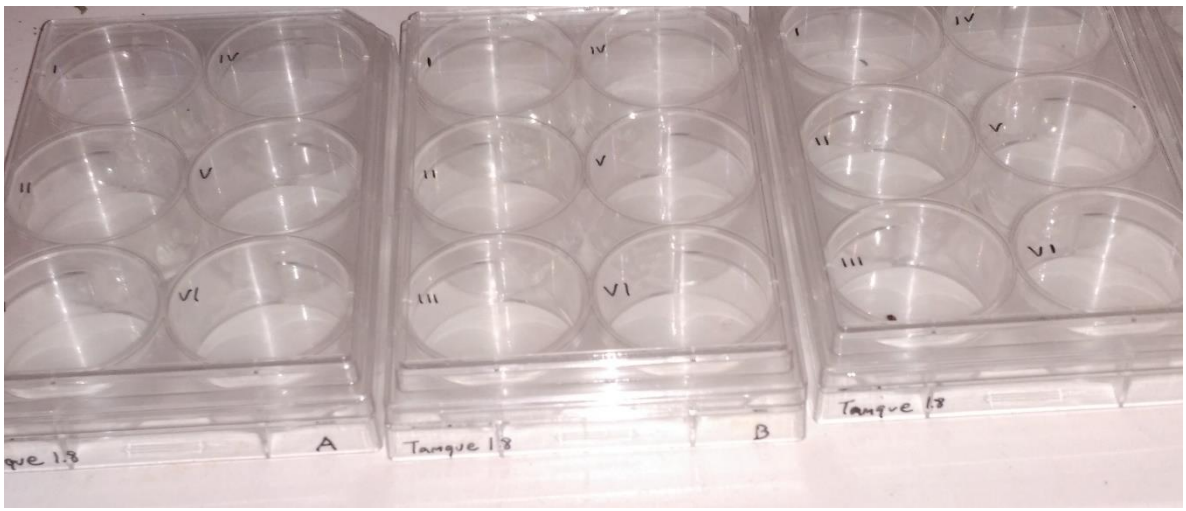

Figure 18: Detail of the specimen plate tagging. These plates were tagged accounting for all culture tanks (photo taken by André Rocha).

The water used in the experiment was prepared by mixing 35 g of Pro Reef™ pharmaceutical grade sea salt for every liter of RO water thus achieving a salinity of 35 ppt and kept at 26 ºC. The larval rearing system was set up as per what was described by Calado et al, 2003a and Calado et al, 2008. The tank model chosen was the cylindrico-spherical tank (a cylindrical tank with a spherical bottom). The six tanks were set up in a recirculation regime and the tank model was chosen for the afore mentioned advantages of this choice of tank ensuring a better suspension of both larvae and prey items through the upwelling water motion and, thus,

avoiding any tangling and damages to certain larval structures if the larvae were to sink or be kept in suspension through other means such as aeration.

The fiberglass cylindrico-spherical tanks measuring 0.35 m in diameter, 0.35 m total height, a spherical bottom 0.22 m from the top and a 20 L capacity were assembled in parallel according to a recirculation system. See Figure 6. The drainage valve on the bottom of each tank as described by Calado et al, 2003a was removed to avoid any disruption to the upwelling phenomenon heightened by the tanks' configuration. Round Nitex™ filters measuring 90 mm 200 mm in diameter with a 500 µm 150 µm mesh respectively were placed 0.1 m below water level and connected to an outlet 25 mm in diameter and equipped with a PVC T 50 mm away from the upper edge of the tank. Figures 18 and 19 illustrate this.

Figure 19: Larviculture system. Notice the sump underneath to the left (photo taken by André Rocha).

Figure 20: Notice the mesh filters and how the larger mesh is above the water since artemia is not being siphoned out. The water inflow pipe valve is also visible, as well as the osmorregulator console behind the tank (photo taken by André Rocha).

The mesh in use will be dependent on the type of prey item used, the smaller mesh being used for smaller prey, such as rotifers, and the larger one for larger prey items, such as *Artemia* nauplii. In this particular experiment, smaller prey items, such as the afore mentioned rotifers, were not used just as *Artemia* enrichment was not used in an attempt to cut down operating costs. These filters are set up to prevent loss of larvae while removing uneaten prey items and, thus, minimizing the needless stress that manually combing the tank for uneaten prey would cause. For this experiment, only the larger mesh filter was used to remove uneaten prey.

The recirculation system was composed by a 243 L sump (0.90 m long, 0.45 m wide and 0.60 m tall) with a biological medium (bioballs) to ensure biological filtration by certain bacteria, a protein skimmer and a pump. This pump sent the water up to a 160 L head tank (0.80 m long, 0.40 m wide and 0.50 m tall) at a rate of 3400 L h^{-1} to then be distributed through gravity by the culture tanks via a ramified PVC (Polyvinyl Chloride) pipe, measuring 20 mm in diameter. The water was then returned to the sump through PVC pipes, measuring 40 mm in diameter, after passing through the Nitex™ mesh filters. Like with the broodstock maturation system, an osmorregulator was set up to ensure the salinity did not fluctuate, see figures 9, 10 and 11. However, given the set-up, a drum could not be used and as such the RO water reservoir was a neighboring tank installed on ground level. A filtration bag like the one used in the broodstock maturation system was fitted to the end of the return pipe to prevent *Artemia* nauplii from spreading across the system when siphoning one-day old uneaten nauplii.

Above the tanks on the ramified supply pipe, valves were fitted on the inflow pipes (one for each inflow pipe) to regulate the flow of water into the culture tanks.

Every larva used in the experiment was captive bred using six breeding pairs for each of the species, *Lysmata uncicornis* e *L. seticaudata*. The progeny thus obtained was used for the starvation trials and the cultivation effort. Amongst the obtained larvae, the ones exhibiting the strongest phototactic response were selected and moved to the larval rearing tanks at a density of 10 larvae L^{-1}, meaning 200 larvae per replica in each 20 L culture tank. Every other day, a small number of larvae was selected randomly for staging. The animals were fed once a day with a possibility for a second feeding when closer to their estimated time of metamorphosis into the post larva stage. Figures 21 and 22 illustrate this.

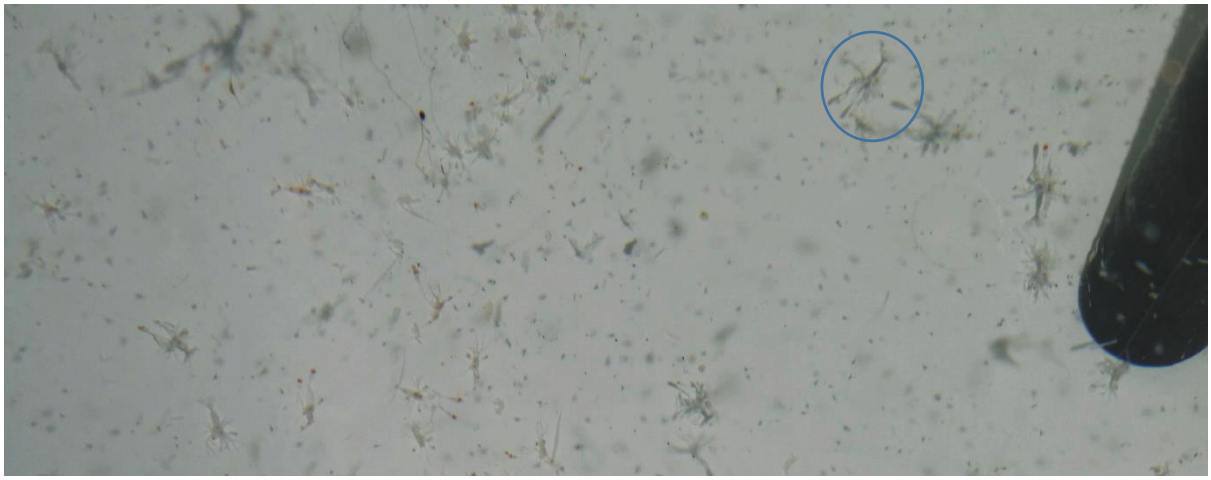

Figure 21: *Lysmata seticaudata* larvae with *Artemia* nauplii in a culture tank. The blue circle highlights one of the larvae (photo taken by André Rocha).

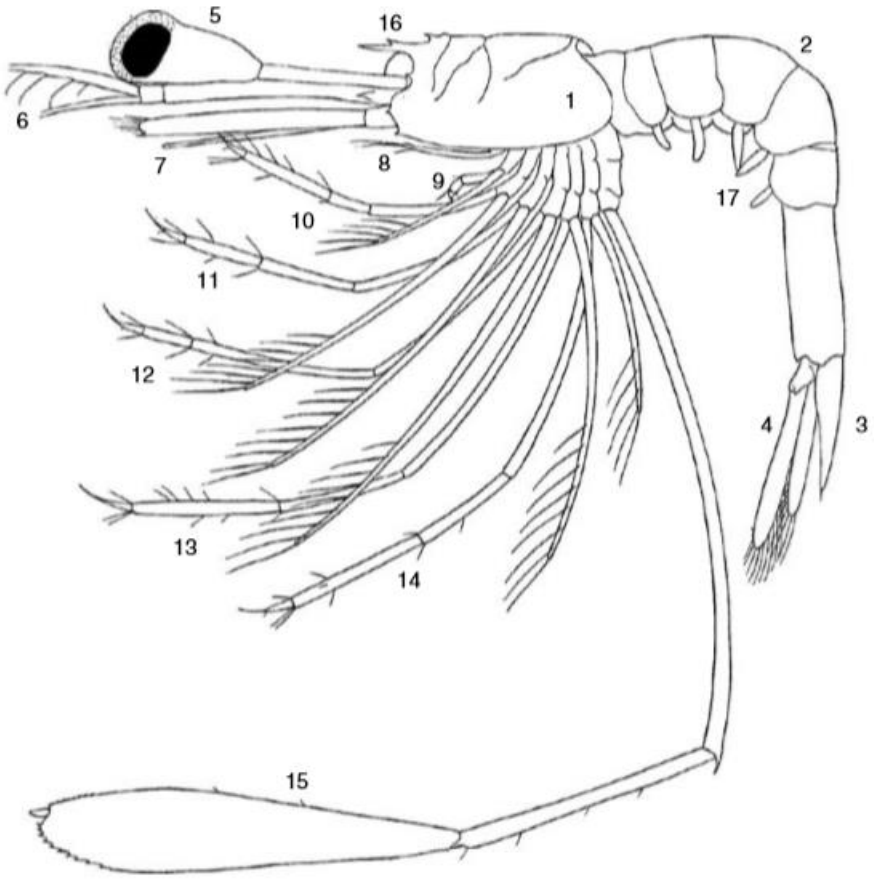

Figure 22: General larval morphology of a caridean zoeal stage using a 'typical' Lysmata larvae as an example: 1, carapace; 2, abdominal segments; 3, telson; 4, uropods (inner and outer branches); 5, stalked eye (fused with the carapace in newly hatched larvae); 6, antennules; 7, antenna; 8, first maxilliped; 9, second maxilliped (note exopod displaying long natatory setae); 10, third maxilliped (note exopod displaying long natatory setae); 11, first pereiopod (note exopod displaying long natatory setae); 12, second pereiopod (note exopod displaying long natatory setae); 13, third pereiopod (note exopod displaying long natatory setae); 14, fourth pereiopod (note exopod displaying long natatory setae); 15, fifth pereiopod (Lysmata larvae display this appendage elongated with a flattened paddle-like propodus); 16, rostrum (may already display rostral spines); 17, pleopods (these structures never display natatory functions in zoeal stages) (adapted from Calado, 2008d)

The larvae were fed *Artemia* nauplii (INVE®) hatched on location using a cylindrical vat with a conical bottom heated o 26 ºC and kept in suspension through aeration by means of an air pump. The nauplii were separated from the hatched and unhatched cysts using a magnet to capture the afore mentioned cysts that were impregnated with iron particles and the nauplii were then siphoned using an aeration tube. Every day before feeding the larvae, five samples were taken and screened for the number of hatched, unhatched and newly hatched nauplii per mL with the highest and lowest recordings being discarded and the density per mL being estimated by calculating the average of the remaining three recordings. The larvae were fed with a density of three nauplii per mL, meaning that a total of 60 000 nauplii were supplied per each 20 L culture tank. Newly hatched *Artemia* was favored and, thus, one-day old nauplii were removed via filtration (using the larger mesh filter) and replaced with freshly hatched ones. Excess hatched artemia was redirected to the feeding of corals pertaining to another

experiment occurring in an adjacent room rather than being discarded. Figures 23 and 24 show the hatching and harvesting processes.

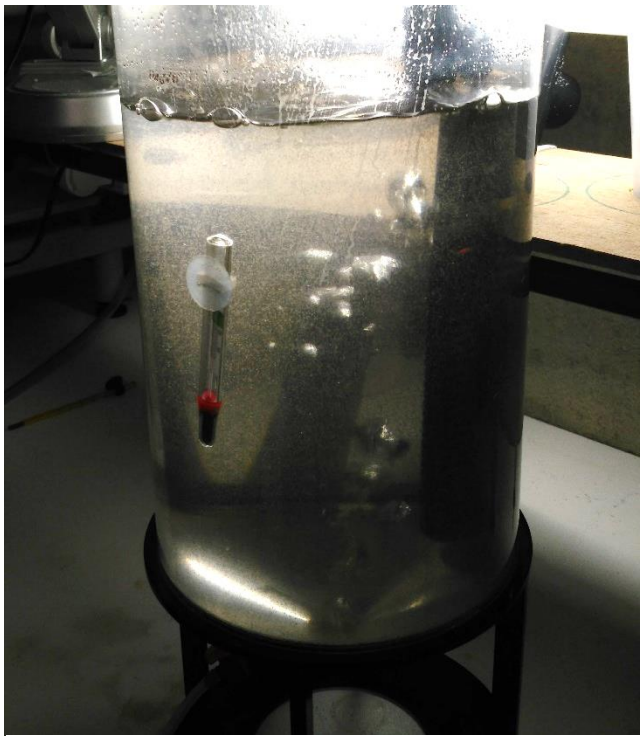

Figure 23: *Artemia* hatching vat with a conical bottom to better ensure the suspension of cysts through aeration (photo taken by André Rocha).

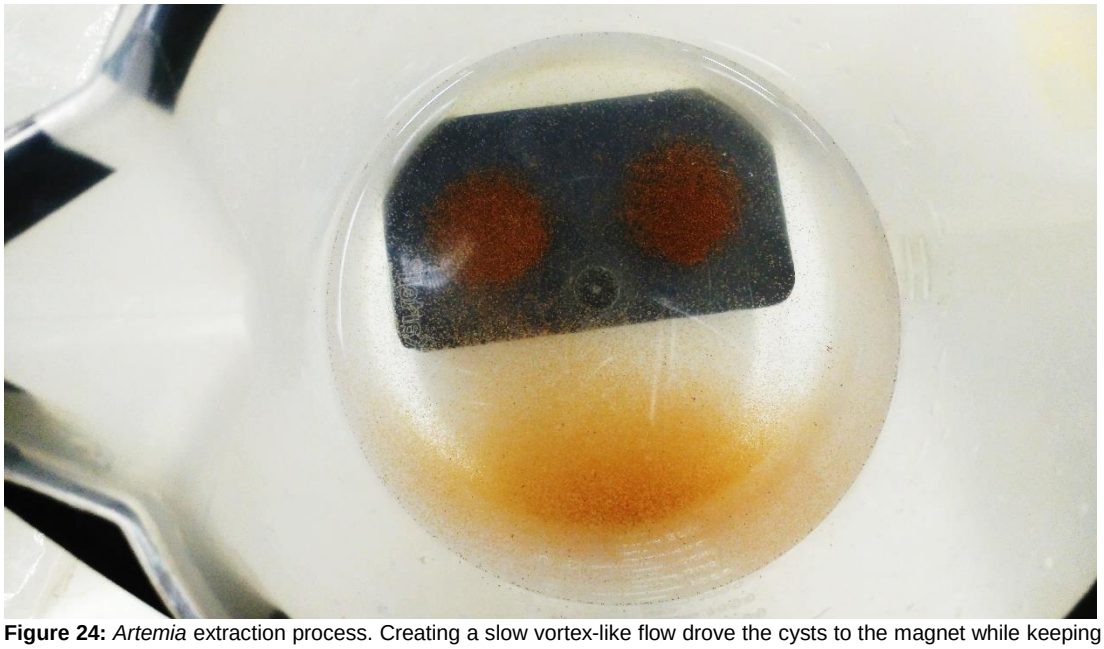

Figure 24: *Artemia* extraction process. Creating a slow vortex-like flow drove the cysts to the magnet while keeping the nauplii suspended. The nauplii would then be siphoned out and washed with salt water before being fed to the larvae (photo taken by André Rocha).

2.1.3. Juvenile Grow-out

After larviculture was concluded, the animals that made it to the postlarval stage were then transferred to the upper level of the maturation system and fed the same diet as the adults, one meal of formula feed and four others of the seafood mix practical diet in two-hour intervals (same regime as the broodstock) until they reached the market size set at 25 mm (total length).

2.2. Economic Feasibility of *Lysmata spp.* Aquaculture

The production and maintenance costs were accounted for and tallied in an effort to determine which of the species was best suited to a mass rearing effort aimed at the aquarium trade industry. Such costs include electricity and water expenses, the salt (Pro Reef™ pharmaceutical grade salt for modern reef aquaria) to prepare the artificial salt water, the tanks themselves along with the pumps, skimmers, pipes and frames to build the maturation and larviculture systems, the air pump and vat to hatch the *Artemia* nauplii, the lighting used, the various feeds (formula feed and practical feeds for the broodstock and *Artemia* for the larvae), the animals that were housed as breeding pairs (14 *L. uncicornis* and 14 *L. seticaudata* individuals), the filter and divisor meshes and rent. These costs were then summed up and matched up against the income that the sales of these animals would generate at current rates considering the survival rates obtained with the experiment. One year of production was analyzed considering that each animal releases larvae twice each month.

2.3. Risk Analysis of *Lysmata spp.* Aquaculture

A SWOT analysis was performed to assess the merits and shortcomings of *Lysmata uncicornis* when compared to the more established *L. seticaudata* in terms of fitness and performance providing insight on areas that may require attention given their potential to compromise the enterprise.

2.4. Statistical Analysis

The data obtained with the starvation trial, breeding effort, larvae cultivation and juvenile grow out was treated and analyzed utilizing tools and functions provided by Microsoft's Excel software before proceeding to the statistical analysis by means of Student's T-tests, after running a Levene Test to assess equality of variances in each case, using SPSS statistical software.

There were seven null hypotheses that were tested for significance ($p \leq 0.05$) by means of a Student's T- test. For the starvation test the null hypothesis was that there was no difference in the number of days that it took the larvae die entirely. To this end, the number of dead animals per day was calculated and, from these numbers, the average days it took for a larva to die and its standard deviation were thus obtained and then used in a Student's T-test. The other null hypothesis was that there were no differences in how capable the larvae were to molt to Zoea II with no food between both species. To achieve this, the number of molts per day were calculated along with the average time in days it took for a larva to change from Zoea I to Zoea II and its standard deviation. The values obtained were then used in a Student's T-Test. Regarding the breeding effort, the null hypothesis was that there were no differences in performance regarding both species' larvae production per animal so, the average larvae production per animal and its standard deviation were calculated and then a Student's T-Test was run. In regard to the larvae cultivation effort, the null hypothesis was that there was no difference in survival rates between each species so, the average survival rate and its standard deviation were calculated for each species from the three replicas and then a Student's T-Test was run thus proving or disproving the null hypothesis. The time the larvae spent in their larval stages was also assessed with the null hypothesis being that there were no significant differences between the periods the animals spent in their larval stages. The average time in days and its standard deviation were calculated and a Student's T-Test was run. Finally, for juvenile grow-out, the null hypotheses were that there were no significant differences between the average time spent in between hatching and the set market size for both species and that there were no significant differences between juvenile survival upon reaching the set market size so the average survival rates and respective standard deviations upon reaching market size were calculated and a Student's T-Test was run.

2.5. Ethical Disclaimer

All experiments were performed taking into account national and EU regulations on animal experimentation and welfare.

3. Results

3.1. *Lysmata spp.* Aquaculture

3.1.1. Broodstock Husbandry and Maturation

The breeding effort was largely successful with both species' broodstock regularly producing larvae. *Lysmata uncicornis* generated a minimum of 124 larvae per individual, a maximum of 1463 larvae per individual and an average of 753±440 larvae per individual. *L. seticaudata* generated a minimum of 118 larvae per individual, a maximum of 610 larvae per individual and an average of 257±123 larvae per individual. There were, however, losses in the broodstock, particularly with *L. uncicornis* where total loss of broodstock was recorded. *L. seticaudata* however had only one recorded death in its broodstock over the course of the experiment. A Levene test for equality of variances was run showing equality of variance and the independent T test (95% Confidence Interval) showed that there was a significant difference, $t(61)=7.082$, with $p<0.0001$ therefore rejecting the null hypothesis. *L. uncicornis* does generate more larvae per animal than *L. seticaudata*. Tables 1 and 2 illustrate this result.

	V2	N	Mean	Standard Deviation	Mean Standard Error
V1	*L. uncicornis*	15	753	440	114,6783
	L. seticaudata	48	257	123	17,7248

Table 1: Mean larvae release size per broodstock animal and its standard deviation.

		Levene Test for Equality of Variances		T-Test for Equality of Means						
								Difference	95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (bilateral)	Mean Difference	Standard Error	Inferior	Superior
V1	Equal Variances Assumed	41.262	.000	7.082	61	.000	496.1875	70.0670	356.0799	636.2951
	Equal Variances Not Assumed			4.313	14.686	.001	496.1875	115.0519	250.5034	741.8716

Table 2: T-Test comparing both species' fertility (number of larvae per animal per release).

3.1.2. Larval Quality and Larviculture

The starvation test showed that the time that a *Lysmata uncicornis* larva took to die averaged at 5.5±1.8 days and took a total of 9 days until all the 300 larvae used in the experiment were dead. As for *L. seticaudata*, the time a larva took to die averaged at 7.1±1.5 days and it took a total of 10 days until all the 300 larvae used in the experiment were dead. A Levene Test for equality of variances was run showing equality of variances and the independent T test (95% Confidence Interval) showed that there was a significant difference, t(598)= -12.097, with p<0.0001 and therefore rejecting the null hypothesis. *L. uncicornis* larvae do die faster than *L. seticaudata* when starved. Tables 3 and 4 better illustrate this.

	V2	N	Mean	Standard Deviation	Mean Standard Error
V1	*L.uncicornis*	300	5.5	1.8	.101
	L.seticaudata	300	7.1	1.5	.087

Table 3: Mean time it takes for a larva to die and respective standard deviation.

		Levene Test for Equality of Variances		T-Test for Equality of Means						
		F	Sig.	t	df	Sig. (bilateral)	Mean Difference	Difference Standard Error	95% Confidence Interval Difference Inferior	Superior
V1	Equal Variances Assumed	12.682	.000	-12.097	598	.000	-1.613	.133	-1.875	-1.351
	Equal Variance Not assumed			-12.097	585.378	.000	-1.613	.133	-1.875	-1.351

Table 4: T-test comparing both species mean survival times per larva when starved.

The test also showed that the time that a *L. uncicornis* larva took to molt into Zoea II when starved averaged at 4.3±1.6 days and it took a total of 6 days until all 142 larvae that made the change reached Zoea II. As for *L. seticaudata*, the time it took for a larva to molt into Zoea II averaged at 3.4±0.7 days and it took a total of 5 days until all 268 larvae that made the change reached Zoea II. A Levene Test for equality of variances was run showing equality of variances and the independent T test (95% Confidence Interval) showed that there was a

significant difference, t(408)=8.033, with p<0.0001 therefore rejecting the null hypothesis. *L. seticaudata* larvae not only made the change faster, more of them did it, 268 out of 300 as opposed to 142 *L. uncicornis* larvae out of 300. Tables 5 and 6 and Figures 25 and 26 better illustrate this.

	V2	N	Mean	Standard Deviation	Mean Standard Error
V1	*L.uncicornis*	142	4.3	1.6	.134
	L.seticaudata	268	3.4	.7	.045

Table 5: Mean time it takes for a larva to molt to Zoea II and respective standard deviation.

		Levene Test for Equality of Variance		T-Test for Equality of Means						
		F	Sig.	t	df	Sig. (bilateral)	Mean Difference	Difference Standard Error	95% Confidence Interval Difference Inferior	Superior
V1	Equal Variances Assumed	218.823	.000	8.033	408	.000	.929	.116	.702	1.156
	Equal Variances Not Assumed			6.559	173.487	.000	.929	.142	.649	1.208

Table 6: T-test comparing both species mean time until molting into Zoea II per larva when starved.

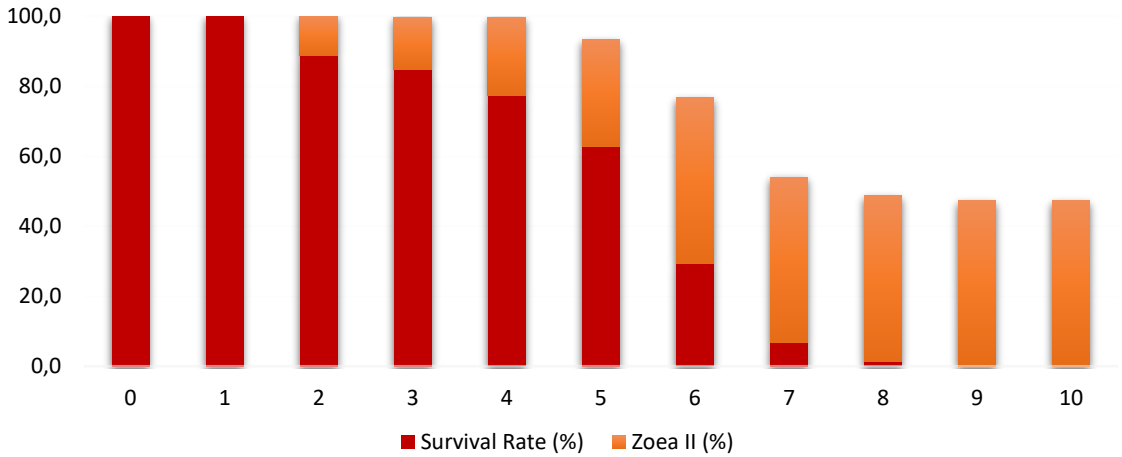

Figure 25: Evolution of the survival rate and Zoea II numbers of *Lysmata uncicornis* over the course of the starvation trial.

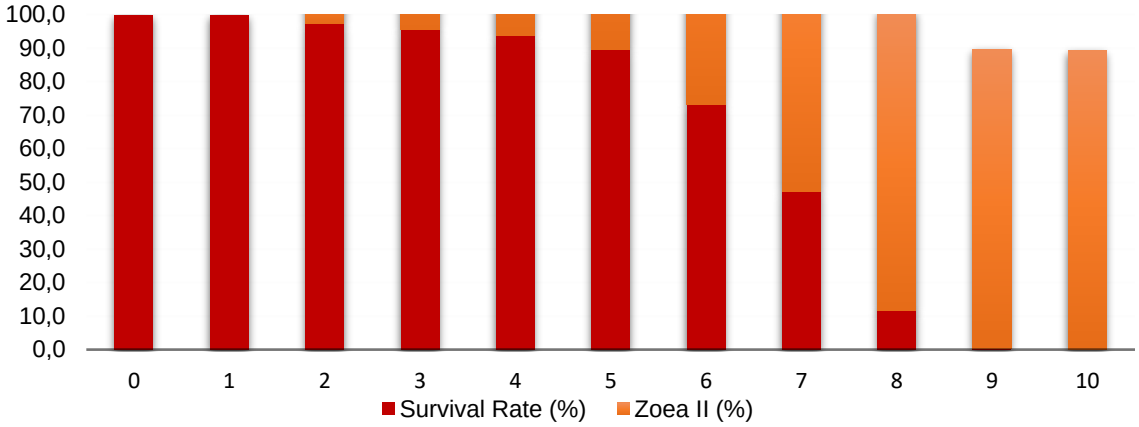

Figure 26: Evolution of the survival rate and Zoea II numbers of *Lysmata seticaudata* over the course of the starvation trial.

The larviculture effort for *L. uncicornis* yielded an average survival of 8±2.0% (out of 200 larvae per replicate) to the post larva phase. The larviculture effort for *L. seticaudata* yielded an average survival of 6.5±1.5 (out of 200 larvae) to the post larva phase. A Levene Test for equality of variances was run showing that there was no equality of variances and the independent T test (95% Confidence Interval) showed that there were no significant differences between larval survival displayed by both species, t(3.709)= 1.039, with p>0.05 (p=0.362) therefore accepting the null hypothesis. Tables 7 and 8 better illustrate this.

	V2	N	Mean	Standar Deviation	Mean Standard Error
V1	*L.uncicornis*	3	8.0	2.0	1.1547
	L.seticaudata	3	6.5	1.5	.8660

Table 7: Mean survival rate for both species and respective standard deviation.

		Teste de Levene para igualdade de variâncias		T-Test for the Equaliy of Means						
									95% Difference Confidence Interval	
		F	Sig.	t	df	Sig. (bilateral)	Mean Difference	Difference Standard Error	Inferior	Superior
V1	Equal Variances Assumed	.160	.710	1.039	4	.357	1.5000	1.4434	-2.5075	5.5075
	Equal Variances Not Assumed			1.039	3.709	.362	1.5000	1.4434	-2.6345	5.6345

Table 8: T-Test comparing both species mean survival rates.

The time *L. uncicornis* larvae spent in their larval stages averaged at 25.3±0.6 days whereas *L. seticaudata* spent an average of 28.0±4.0 in their larval stages. A Levene Test for equality of variances was run showing that there was no equality of variances and the independent T-test (95% Confidence Interval) showed that there were no significant differences between the species regarding time spent in their larval stages, t(2.083)=-1.143, with p>0.05 (p=0.367) thus accepting the null hypothesis. Tables 9 and 10 better illustrate this.

	V2	N	Mean	Standard Deviation	Mean Standard Error
V1	*L.uncicornis*	3	25.3	.6	.333
	L.seticaudata	3	28.0	4.0	2.309

Table 9: Mean time spent during larval stages for both species and respective standard deviation.

		Levene Test for the Equality of Variances		T-Test for the Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (bilateral)	Mean Difference	Standard Error Difference	Inferior	Superior
V1	Equal Variances Assumed	2.759	.172	-1.143	4	.317	-2.667	2.333	-9.145	3.812
	Equal Variances Not Assumed			-1.143	2.083	.367	-2.667	2.333	-12.331	6.998

Table 10: T-test comparing mean time spent in larval stages for both species.

3.1.3. Juvenile Grow-out

The time it took for *L. uncicornis* to reach their market size (from larval hatching to 25 mm total length at juvenile stage) averaged at 84±6.0 days whereas *L. seticaudata* larvae took 85±5.0 days to reach the same state. A Levene Test for equality of variance was run showing that there was no equality of variance and the independent T test (95% Confidence Interval) showed that there were no significant differences between the two species regarding the time it takes for the animals to reach market size, starting to mark the time since the animals' hatching, t(3.874)=-0.222, with p>0.05 (p=0.836) therefore accepting the null hypothesis. Tables 11 and 12 better illustrate this.

	V2	N	Mean	Standard Deviation	Mean Standard Error
V1	*L.uncicornis*	3	84.0	6.0	3.464
	L.seticaudata	3	85.0	5.0	2.887

Table 11: Mean time it takes for the larvae to reach market size as juveniles and respective standard deviation.

		Levene Test for the Equality of Variances		T-test for the Equality of Means						
									95% Difference Confidence Interval	
		F	Sig.	t	df	Sig. (bilateral)	Mean Difference	Difference Standard Error	Inferior	Superior
V1	Equal Variances Assumed	.066	.811	-.222	4	.835	-1.000	4.509	-13.520	11.520
	Equal Variances Not Assumed			-.222	3.874	.836	-1.000	4.509	-13.682	11.682

Table 12: T-test comparing the mean time it takes for the larvae to reach market size as juveniles for both species.

L. uncicornis survival at market size averaged at 61±15.1% whereas L. seticaudata survival averaged at 67.33±3.786%. A Levene Test for equality of variances was run showing that there was no equality of variances and the independent T test (95% Confidence Interval) showed that there were no significant differences between the species' survival rates upon reaching market size, t(2.250)=-0.705, with p>0.05 (p=0.547), therefore accepting the null hypothesis Tables 13 and 14 better illustrate this.

	V2	N	Mean	Standard Deviation	Mean Standard Error
V1	*L.uncicornis*	3	61.0	15.1	8.718
	L.seticaudata	3	67.3	3.8	2.186

Table 13: Mean survival rates for juvenile shrimp upon reaching market size and respective standard deviations.

		Levene Test for Equality of Variances		T-Test for Equality of Means						
									95% Difference Confidence Interval	
		F	Sig.	t	df	Sig. (bilateral)	Mean Difference	Difference Standard Error	Inferior	Superior
V1	Equal Variances Assumed	3.068	.155	-.705	4	.520	-6.333	8.988	-31.287	18.620
	Equal Variances Not Assumed			-.705	2.250	.547	-6.333	8.988	-41.159	28.492

Table 14: T-test comparing mean survival rates for juveniles upon reaching market size.

3.2. Economic Feasibility of *Lysmata spp.* Aquaculture

Considering survival rates of 8% for *L. uncicornis* and 6.5% for *L. seticaudata*, juvenile survival rates of 61% for *L. uncicornis* and 67.33% *for L. seticaudata* and that each animal releases larvae twice a month for 12 months, totals of 12 347 *L. uncicornis* juveniles and 3 779 *L. seticaudata* juveniles could be obtained. At a selling price of 5 € per animal this would sum up to 80 629.71 € and thus covering the total expense costs. Table 15 better illustrates these calculations.

Cost ID	Cost Type	Unit	Quantity	Unit Cost	Cost Value (€)	Depreciation
Broodstock maturation system	Fixed	unit	1,00	2500,00	**312,50**	8 years
Larvae cultivation system	Fixed	unit	1,00	1500,00	**187,50**	8 years
Broodstock - *Lysmata uncicornis*	variable	individual	14,00	8,00	28,00	4 years
Broodstock - *Lysmata seticaudata*	variable	individual	14,00	8,00	28,00	4 years
Total Broodstock costs	variable	individual	28,00	8,00	**56,00**	4 years
Feeding - Hikari formula feed	variable	kg	0,50	50,00	25,00	
Feeding - Seafood mix	variable	kg	4,00	6,00	24,00	
Feeding - Artemia	variable	kg	1,00	100,00	100,00	
Total feeding costs	variable	kg			**149,00**	
Broodstock System Water	variable	m3	3,24	0,45	1,46	
Larviculture System Water	variable	m3	2,09	0,45	0,94	
Water changes - Broodstock system	variable	m3	7,14	0,45	3,21	
Water changes Larviculture system	variable	m3	4,60	0,45	2,07	
Water - Staff consumption	variable	m3	12,00	0,45	5,39	
Total water consumption		m3	29,08	0,45	13,07	
Sea Salt	variable	kg	830,72	5,00	4 153,60	
Total saltwater costs	variable				**4 166,67**	
Electricity - Broodstock pumps (2)	variable	kWh	2190,00	0,22	473,71	
Electricity - Larviculture pumps (1)	variable	kWh	700,80	0,22	151,59	
Electricity - Broodstock thermostat (2)	variable	kWh	2628,00	0,22	568,46	
Electricity - Larviculture thermostat (1)	variable	kWh	1314,00	0,22	284,23	
Electricity - *Artemia* vat thermostat (1)	variable	kWh	1314,00	0,22	284,23	
Electricity - *Artemia* vat air pump (1)	variable	kWh	219,00	0,22	47,37	
Electricity - Skimmer pumps (2)	variable	kWh	1016,16	0,22	219,80	
Electricity - Lighting (2)	variable	kWh	210,24	0,22	45,48	
Total Electricity costs	variable	kWh	9592,20	0,22	**2 074,86**	
Wages	fixed	€	14,00	1000,00	14 000,00	
Social Labor Costs	fixed	€	14,00	250,00	3 500,00	
Total Labor Costs	fixed	€	14,00	1250,00	**17 500,00**	
Rent (month)	fixed	m2	50,00	6,80	340,00	
Rent (year)	fixed	m2	50,00	6,80	**4 080,00**	
Total operating cost	variable	€			**28 526,53**	

Table 15: Cost assessment of the *Lysmata spp* aquaculture enterprise after a year functioning.

3.3. Risk Analysis of *Lysmata spp.* Aquaculture

In light of the results that were obtained, a SWOT (Strengths, Weaknesses, Opportunities and Threats) matrix was drawn up to better illustrate one species' performance over the other, in this case *Lysmata uncicornis* over *L. seticaudata* regarding the possibility of one or the other being mass produced in captivity with the target market being the marine aquarium trade. For Strengths:

- Can easily mature in captivity;
- Greater number of larvae per specimen;
- Can be cultured using protocols already well-established for a related species (*L. seticaudata*);
- Higher market price than concurrent species (*L. seticaudata* and *L. wurdemanni*).

For Weaknesses:

- Poor acclimation capacity to captivity of wild broodstock;
- High intraspecific aggression of broodstock in captivity;
- Specific nutritional requirements still unclear.

For Opportunities:

- Novel species in the trade;
- More appealing coloration;
- Specimens born in captivity are better suited to it and perform better;
- Alleviating pressure on wild stocks.

For Threats:

- Better culture performance of *L. seticaudata;*
- End-consumers likely reluctant to acquire both species;
- More expensive than wild-caught specimens.

Table 16 helps to better visualize this analysis.

S Can easily mature in captivity Greater number of larvae per specimen Can be cultured using well-established protocols Higher price than concurrent species	**W** Poor acclimation capacity to captivity of wild broodstock High intraspecific aggression of broodstock in captivity Specific nutritional requirements still unclear
O Novel species in the trade More appealing coloration Cultured specimens perform well in captivity Alleviate pressure on wild stocks	**T** Better culture performance of *L. seticautada* End-costumers likely reluctant to acquire both species More expensive than wild-caught specimens

Table 16: SWOT matrix analyzing *Lysmata uncicornis* Strengths, Weaknesses, Opportunities and Threats when compared to *L. seticaudata*.

4. Discussion

4.1. Broodstock Housing and Maturation

The breeding effort yielded satisfactory results with *L. uncicornis* producing an average of 753 larvae (with a standard deviation of 440) per animal, considering that these animals can generate up to around 1000 larvae per broodstock animal and the release is dependent on the size of the ovigerous shrimp (Ricardo Calado, personal communication). *Lysmata seticaudata* did not perform as well generating on average 257 larvae (with a standard deviation of 122). However, taking into account that these animals typically produce 150 to 500 eggs per animal (Lagardère, 1971), the size of *L. seticaudata* releases, though not ideal, were not at all disheartening. The diet provided to the animals also could account for a less than ideal release size. A lipid deficient diet could contribute for poor gonad maturation, thus compromising the shrimps' fertility. The use of live food items instead of the practical feed (frozen seafood mix) used in the experiment could have contributed to better satisfy these animals' dietary needs. To that end, prey item enrichment using the EFA (Essential Fatty Acids) LOA (Linoleic Acid), LNA (Linolenic Acid), ARA (Arachidonic Acid), EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid) presents itself as a viable solution to this potential deficiency. An example of such practice is the lipid enrichment of rotifers and artemia nauplii. Although these prey items are not part of these animals' natural diet, they exhibit an adequate nutritional profile that, once supplemented, presents itself as appropriate for the diets of both larvae and mature breeding adults (Tziouveli, Hall e Smith, 2012). This however may not have been too significant since the broodstock of both species was fed the same diet (quantitatively and qualitatively) five times per day, using the same seafood mix and on the same two-hour intervals with the animals successfully achieving gonad maturation and regularly releasing larvae.

To ensure the commercial viability of an ornamental species it is necessary to produce high quality larvae in large numbers and on a regular basis, thus, beyond formulating adequate diets that satisfy the larvae's needs, it is also of paramount importance that the breeding pairs' diet is the best it can possibly be during and after their maturation (Olivotto et al, 2011). Analyzing the particular case of the genus *Lysmata*, a genus whose species are all protandric simultaneous hermaphrodites, it is doubly important that the breeding pairs are kept on an adequate diet since each individual has to bear the energy expenditure of both oogenesis and spermatogenesis (Calado et al, 2009) and thus ensure the production of good quality gametes and, consequently, good quality larvae.

A total loss of the *L. uncicornis* broodstock was recorded. This should not be attributed to water quality issues since not all animals died at the same day (or only a few days apart) and the system water was tested with colorimetric tests for ammonia, nitrates and nitrites that always displayed values that ranked within the "below-detectable levels" category for all these parameters. Furthermore, only one specimen of *L. seticaudata* died (likely due to agonistic behavior) while sharing the same water of *L. uncicornis* broodstock. The fact that the animals shared the same water in a recirculation regime also rules out diseases. *Lysmata uncicornis* are relatively new to the aquarium trade and acclimate poorly to captivity (Ricardo Calado, personal communication). These features coupled with a potential deficiency in certain types of seaweed in their diets (Lagardère, 1971) might have contributed to the loss of the animals coupled with agonistic behavior towards conspecifics (personal observation).

4.2. Larval Quality and Larviculture

The starvation test indicates that *L. seticaudata* larvae might be more fit than their *L. uncicornis* counterparts by being able to better endure starvation, as on average they took longer to die and were able to molt to Zoea II in larger numbers despite starvation. This assumption is further supported by the fact that the PNR (Point of No Return), meaning the point at which starved larvae even if fed may remain alive for a number of days but the nutritional stress is such that the animal will ultimately die (Calado et al, 2008), is already known not be homogenous across the genus *Lysmata* (Calado et al, 2007a).

Upon hatching these animals carry energy reserves that result from maternal investment that allow them to go without food for a variable period. However, evidence suggests that, despite these energy reserves, these animals are capable to feed upon hatching and, should they have the chance, will do so even if simply by ingesting microalgae (Simões et al, 2002). This feature is termed Facultative Primary Lecithotrophy (Calado et al, 2008) since the animals can indeed feed upon hatching. The longer a larva can go without exogenous feed, the bigger these energy reserves are, making the larva more fit (hence displaying a higher maternal investment). Some species can molt to Zoea II (Figueiredo and Narciso, 2006), however the belief that all the members of the genus can do this is erroneous and has led to larviculture practices that prolong nutritional stress longer than necessary (Calado et al, 2008). This larval feature allows for some food independence in the wild in areas where larval prey availability is heterogenous and can be advantageous in captivity since these animals release their larvae during the night (Lagardère, 1971), which means that there may be a six to twelve-hour period that they will have to endure without food (Calado et al, 2007a; Calado et al, 2008) until the rearing system operator arrives in the following morning. It is, thus, important to reduce as

much as possible the time the animals endure starvation (Simões et al, 2002) because even if the larvae are able to survive these starvation periods, they will negatively affect their performance during their larval stage by extending it through mark time molts, increasing mortality at metamorphosis and causing asynchronous settlements (Calado et al, 2005a; Calado et al, 2005b; Calado et al, 2007a) that can lead to bigger postlarvae and juveniles preying on the other larvae and recently settled postlarvae. Furthermore, over the course of their larval development, these animals can accumulate energy reserves as they feed (Ricardo Calado, personal communication). This feature may allow some species to display Facultative Secondary Lecithotrophy as animals catabolize these energy reserves should an exogenous food source be unavailable (Calado et al, 2007b). This can, in akin to what Facultative Primary Lecithotrophy grant the larvae, lead specimens to extend their larval development. Nonetheless, by prolonging their exposure to nutritional stress larvae may eventually compromise their quality to the point that they may no longer be able to molt to postlarvae (Calado et al, 2010b), ultimately dying before reaching the juvenile stage.

Since *L. uncicornis* is native to warmer climates the water temperature employed (26 ºC) should not have impacted the larvae negatively. However previous work suggests that *L. seticaudata* larvae, with the species being native to more temperate waters, could fare better at 20 ºC (Figueiredo and Narciso, 2006) so temperature also may have contributed to the low survival rate to post larva that the experiment obtained. However, from a commercial scale culture standpoint it is more advantageous to keep the temperature at 26 ºC since this yields a faster larval growth and development (Figueiredo and Narciso, 2006).

The diet provided to the larvae is also very important in their performance. The one big limitation of the more commonly used diets used during larval development such as rotifers and, in this case, *Artemia* nauplii resides in its lipid content, namely PUFA (Poly-Unsaturated Fatty Acids) and HUFA (Highly Unsaturated Fatty Acids). As such, due to crustaceans' inability to synthetize these compounds *de novo* (Calado et al, 2005b; Calado et al, 2010a), the animals fed on these diets will end up suffering from lipid deficiencies. Lipids are an important component in crustacean diets in that these compounds act as energy reserves (triacyclglycerols), cellular membrane components (phospholipids) and as hormonal substrates (steroids) and are mobilized in processes the likes of oogenesis and vitelogenesis (Tziouveli e Smith, 2012). In keeping with what was mentioned before, these animals need an exogenous supply of HUFA (Palmtag e Holt, 2007) due to their low efficiency or even inability to convert 18C PUFA into 20C or 22C HUFA (Calado et al 2005b; Tziouveli, Hall e Smith, 2012). To address these dietary limitations prey item enrichment using the EFA (Essential Fatty Acids) (Díaz-Jiménez et al, 2017) LOA (Linoleic Acid), LNA (Linolenic Acid), ARA (Arachidonic Acid), EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid)

presented itself as a viable solution. An example of such practice is the lipid enrichment of rotifers and *Artemia* nauplii (Díaz-Jiménez et al, 2017). Although these prey items are not part of the larvae's natural diet, they exhibit an adequate nutritional profile that, once supplemented, presents itself as an appropriate profile for the diets of both larvae and mature breeding adults (Tziouveli, Hall e Smith, 2012). Amongst other functions, ARA (predominantly) and EPA share a role that is worthy of note, these fatty acids intervene in the synthesis of prostaglandins and other eicosanoids that, in turn, intervene in reproductive and molting processes, meaning that the adults may not mature properly and, the larvae might undergo mark-time molts in case of deficiencies of these fatty acids, thus demonstrating fatty acids' relevance in crustaceans' diet whether one is addressing breeding pairs or larvae (Tziouveli, Hall e Smith, 2012). The n-3 HUFA EPA and DHA are also particularly relevant to crustaceans owing to their role in female maturation (or female reproductive system development in the case of simultaneous hermaphrodites like the species of the genus *Lysmata*), embryo development and larval survival after leaving the egg (Tziouveli e Smith, 2012).

The concept of carry-over effect (Simith et al, 2013) is relevant to explain the poor survival rates the experiment yielded. The marked difference between crustaceans' pelagic larval phase and benthic juveniles and adults in no way means that there are "new begginings" (Simith et al, 2013; Calado and Leal, 2015) This is to say that the larvae's success is dependent on parental investment that is directly tied to their nutrition. The broodstock nutrition was adequate though not ideal so the larvae could only do so well as that allowed. Then during the larval phase, the nutrition was once more not ideal since plain artemia nauplii are not the most balanced of diets (Calado et al, 2007a; Díaz-Jiménez et al, 2017). Delayed settlement and metamorphosis through mark-time molts have been considered as one of the main causes of early post-larval mortality in most benthic marine invertebrates (Gebauer et al, 2003; Simith et al, 2013). Also, the absence of certain conspecific adult-secreted odors that in the wild would permeate the substrate and act as a cue to metamorphosis (Gebauer et al, 2003) is another plausible explanation for the animals delayed metamorphosis into the post larva phase. This combination of factors is what might have led to the poor survival rates that were recorded after the larviculture experiment ended.

4.3. Juvenile Grow-out

The juveniles performed better than the larvae achieving an average survival at market size (25 mm total length) of 61.0±15.1% for *Lysmata uncicornis* and 67.33±3.786% for *L. seticaudata*. Not only did it seem that at this stage they presented better resilience they also showed signs of gonad maturation and some even became ovigerous at market size (25 mm)

still a much smaller size than what is seen in the wild (Calado et al, 2005b; Calado and Dinis, 2007). The male phase typically undergoes sex change during the second year of life (Coutourier-Bhaud, 1974; Calado et al, 2005b) and for lengths of 40 mm, much later and much bigger than their captive-bred counterparts (Calado and Narciso, 2003; Calado and Dinis, 2007). This can be detrimental to the time the animals are taking to grow since energy that would at that stage be allocated to somatic development is being diverted for sexual maturation (Calado and Dinis, 2007; Ricardo Calado, personal communication). Calado and Dinis (2007) achieved a similar marketable size to the one set for this experiment in two months while the current experiment yielded an average of 84 days (with a standard deviation of 6.0) for *L. uncicornis* and an average of 85 days (with a standard deviation of 5.0) for *L. seticaudata*. Such phenomenon is highly detrimental for the commercial culture of ornamental shrimps sold on a unitary-size basis (Calado and Dinis, 2007). These specimens were fed the same diet as the broodstock and following the same regime (five meals per day with two-hour intervals) so this, despite deleterious to their somatic development, proves the diet's effectiveness in bringing about gonad maturation and egg emission. Still, this just means that the diet is adequate, not ideal and thus, any prior nutritional deficiencies or stress factors that the larvae and the broodstock animals before them had been exposed to will still be conditioning these animals' performance via the carry-over effect (Simith et al, 2013) and will be conditioning these animals' progeny via what's designated transgenerational effect (Giménez, 2006). There appears to be a correlation between rearing density, temperature and precocious sexual change in juvenile shrimp (Calado and Dinis, 2007) so to prevent this and maximize somatic growth, carrying out the juvenile grow-out by housing the juvenile shrimp individually at 26 ºC presents itself as a viable solution to precocious sexual change from the male phase to the simultaneous hermaphrodite in juveniles. Lowering the temperature to 18 ºC also provides the desired result but it directly impairs the rate at with the animals grow (Calado and Dinis, 2007) so that route is to be avoided.

4.4. Economic Feasibility of *Lysmata spp.* Aquaculture

The feasibility of these animals' cultivation is entirely dependent on being able to, at least, break even given the set costs of such and enterprise. As previously stated, a series of costs were tallied and matched up against what income marketable juveniles, at the achieved survival rates, could generate. The survival rates to postlarvae were lower than expected for the reasons detailed above. However even with a less than ideal performance the larvae produced by the broodstock would allow to pay for the system and still have a decent margin of profit. This means that raising the larvae's survivability would be the next logical step of what can be a very lucrative venture. Furthermore, it would even be possible to culture *L.*

uncicornis by itself since the production of 28 animals would yield 123 467.90 € after a year, considering that each broodstock animal releases larvae twice per month and that each juvenile is sold for 5 € per individual, considering the survival rate upon reaching market size. This also shows that, apparently, cultivating *L. uncicornis* alone, following this culture scheme, would prove the more profitable option. The profits of such enterprise would rise further, should one consider the collectors facet of the hobbyists and the fact that *L. uncicornis* is still somewhat of a novelty species to the aquarium trade. This could allow the aquaculturist to charge more for these animals and, thus, further increasing the culture effort's profitability. The maturation, larviculture and broodstock costs appear as less than what would have been originally payed since these items have a life span during which they serve their purpose and as such, to determine yearly costs tied to these items, total cost was divided by the number of years they would be active. See Table 15 for details.

4.5. Risk analysis of *Lysmata spp.* Aquaculture

Despite the feasibility analysis showing that cultivating both animals would be feasible and even profitable there are some cautioning remarks to be done. Namely, that these animals are sympatric in the wild (Ricardo Calado, personal communication). Even though *L. uncicornis* typically favors deeper waters with rocky bottoms for shelter as opposed to the more coastal *L. seticaudata* (Lagardère, 1971), their geographical distribution can overlap. This is to say that these animals can, in some circumstances, share the same habitat and with the genus' propensity to agonistic behavior towards conspecifics, the same behavior towards congeners is not at all farfetched and, as such, a well-informed aquarium keeper might refrain from purchasing both species for the same aquarium, a factor that can hamper sales and the culture effort's profitability.

5. Concluding Remarks

Despite the low survival rates, the present work shows that it would still be economically viable to carry out the cultivation for both *Lysmata* species studied. And, given the novelty of *L. uncicornis* in the aquarium trade, one could even charge more per individual. With that in mind, the values obtained at the set price and considering the survival rates obtained, *L. uncicornis* would be the more profitable species to culture. However, improvements on broodstock and larval nutrition, that appear to have been the major bottlenecks, are warranted. A diet formulated according to wild larvae's biochemical profile could prove, if not close to ideal, a much more suitable broodstock diet and further studies regarding *L. uncicornis* acclimation and nutritional needs are warranted. It is also apparent that cutting costs in larval nutrition can be tremendously deleterious to the cultivation effort so using a more varied diet with newly hatched enriched *Artemia* nauplii, as well as enriched rotifers, and minced squid or codfish eggs closer to the estimated time of metamorphosis into post larvae is highly advisable. Concerning juvenile grow-out, the practical seafood mix diet seemed appropriate, but the same treatment given to the broodstock would likely yield better results and by housing specimens individually, it could be possible to minimize precocious sexual phase change given the deleterious effect this shift promotes on the growth performance of the animals, as energy that should be fueling somatic growth is diverted to fuel oogenesis and spermatogenesis. This housing strategy, however, bears logistics and room availability issues. Overall, the aquaculture of marine ornamental shrimp, particularly those within genus *Lysmata*, continues to be an appealing venture to marine aquarium enthusiasts and young entrepreneurs willing to put in to practice their academic knowledge on this exciting research field.

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